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A TEXT-BOOK

UPON THE

PATHOGENIC BACTERIA

FOR

STUDENTS OF MEDICINE AND PHYSICIANS

BY

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TO

MY HONORED AND BELOVED GRANDFATHER

Mr. Jacob Grim

WHOSE PARENTAL LOVE AND LIBERALITY ENABLED ME TO PURSUE
MY MEDICAL EDUCATION

THIS BOOK IS AFFECTIONATELY DEDICATED

PREFACE TO THE FIFTH EDITION.

The request for a fifth edition of THE PATHOGENIC BACTERIA was followed by a careful analysis of the reviews of past editions, in the hope that it might be possible to improve the work according to the suggestions offered. Accordingly, much new matter has been added and much that was old and worthless eliminated.

The chapters upon infection and immunity, in which subjects the advance of knowledge is rapid, have been entirely rewritten, it is hoped in a more readable and logical manner.

The original plan of limiting the book strictly to the pathogenic bacteria has not been departed from.

According to the conception of the author, every well written text-book must accomplish three purposes; first, that of interesting the reader in the subject with which it deals; second, that of conveying to him all the essential facts of the subject; and third, that of opening to him the literary sources from which the facts are gleaned. One of the reviewers complains that the book is too good from a certain point of view and not good enough from another; that is, as an elementary text-book it is too full and contains too many references to the literature, but to be an encyclopedia it is not full enough. There should be no misunderstanding about the footnotes. They do not, and are not intended to, cover the entire literature of the subjects treated, but simply make accessible the original sources in which important facts, theories, methods and suggestions are contained.

J. McF.

PREFACE.

THE following pages are intended to convey to the reader a concise account of the technical procedures necessary in the study of bacteriology, a brief description of the life-history of the important pathogenic bacteria, and sufficient description of the pathological lesions accompanying the micro-organismal invasions to give an idea of the origin of symptoms and the causes of death.

The work being upon Pathogenic Bacteria, it does not cover the whole scope of parasitology, and the parasites of higher orders are all omitted. Malaria and amebic dysentery are omitted as logically as tape-worms and pediculi. The higher fungi are also omitted, both because they are not bacteria and because their proper consideration would make a small book in itself.

In leaving out the non-pathogenic bacteria of course a stumbling-block was encountered. The Sarcina ventriculi, for instance, may be a cause of dyspepsia, yet can scarcely be regarded as pathogenic, and, together with other similar bacteria of questionable deleterious operation, has been omitted; on the other hand, it has been thought advisable to include and describe somewhat at length a long list of spirilla similar to, and probably closely allied with, the spirillum of cholera, yet not the cause of any particular diseased condition.

The aim has been to describe only such bacteria as can be proven pathogenic by the lesions or toxins which they engender, and, while considering them, to mention as fully as is necessary the species with which they may be confounded.

The book, of course, will find its proper sphere of usefulness in the hands of medical students; its pages, however, will be found to contain much that will be of interest and profit to those practitioners of medicine who graduated before modern science had thrown its light upon the etiology of disease.

In writing this work the popular text-books have been drawn upon. Hüppe, Flügge, Sternberg, Fränkel, Günther, Thoinot and Masselin, and others have been freely consulted.

The illustrations are mainly reproductions of the best the world affords, and, being taken from the great standards, are surely superior to anything new covering the same ground. Credit has carefully been given for each illustration.

J. McF.

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PATHOGENIC BACTERIA.

PART I. GENERAL CONSIDERATIONS.

INTRODUCTION.

Biology, chemistry, medicine, and surgery, in their evolution, contributed to a new branch of knowledge whose subsequent development has been of inestimable importance to each. Indeed, bacteriology illustrates the old adage, "The child is father of the man," for while it is in part the offspring of the medicine of the past, it has established itself as the dictator of the medicine of the present and future, especially so far as concerns the infectious diseases.

THE EVOLUTION OF BACTERIOLOGY.

I. BIOLOGIC CONTRIBUTIONS; THE DOCTRINE OF SPONTANEOUS GENERATION.

Among the early Greeks we find that Anaximander (43d Olympiad, 610 B. C.) of Miletus held the theory that animals were formed from moisture. Empedocles of Agrigentum (450 B. C.) attributed to spontaneous generation all the living beings which he found peopling the earth. Aristotle (384 B. C.) is not so general in his view of the subject, but asserts that "*sometimes* animals are formed in putrefying soil, sometimes in plants, and sometimes in the fluids of other animals."

Three centuries later, in his disquisition upon the Pythagorean philosophy, we find Ovid defending the same doctrine of spontaneous generation, while in the Georgics Virgil gives directions for the artificial production of bees.

The doctrine of spontaneous generation of life was not only current among the ancients, but we find it persisting through the Middle Ages, and descending to our own generation. In 1542, in his treatise called "De Subtilitate," we

find Cardan asserting that water engenders fishes, and that many animals spring from fermentation. Van Helmont gives special instructions for the artificial production of mice, and Kircher in his "Mundus Subterraneus" (chapter "De Panspermia Rerum") describes and *actually figures* certain animals which were produced under his own eyes by the transforming influence of water on fragments of stems from different plants.*

About 1686, Francesco Redi seems to have been the first to doubt that the maggots familiar in putrid meat arose *de novo:* "Watching meat in its passage from freshness to decay, prior to the appearance of maggots, he invariably observed flies buzzing around the meat and frequently alighting on it. The maggots, he thought, might be the half-developed progeny of these flies. Placing fresh meat in a jar covered with paper, he found that although the meat putrefied in the ordinary way, it never bred maggots, while meat in open jars soon swarmed with them. For the paper he substituted fine wire gauze, through which the odor of the meat could rise. Over it the flies buzzed, and on it they laid their eggs, but the meshes being too small to permit the eggs to fall through, no maggots generated in the meat; they were, on the contrary, hatched on the gauze. By a series of such experiments Redi destroyed the belief in the spontaneous generation of maggots in meat, and with it many related beliefs."

In 1683 Anthony van Leeuwenhoek, justly called the "Father of microscopy," demonstrated the continuity of arteries and veins through intervening capillaries, thus affording ocular proof of Harvey's discovery of the circulation of the blood; *discovered bacteria,* seeing them first in saliva, discovered the rotifers, and first saw the little globules in yeast which Latour and Schwann subsequently proved to be plants.

Leeuwenhoek involuntarily reopened the old controversy about spontaneous generation by bringing forward a new world, peopled by creatures of such extreme minuteness as to suggest not only a close relationship to the ultimate molecules of matter, but an easy transition from them.

In succeeding years the development of the compound microscope showed that putrescent infusions, both animal and vegetable, teemed with minute living organisms.

* See Tyndall: "Floating Matter in the Air."

Abbé Lazzaro Spallanzani (1777) filled flasks with organic infusions, sealed their necks, and, after subjecting their contents to the temperature of boiling water, placed them under conditions favorable for the development of life, without, however, being able to produce it. Spallanzani's critics, however, objected to his experiment on the ground that air is essential to life, and that in his flasks the air was excluded by the hermetically sealed necks.

Schulze (1836) set this objection aside by filling a flask only half full of distilled water, to which animal and vegetable matters were added, boiling the contents to destroy the vitality of any organisms which might already exist in them, then sucking daily into the flask a certain amount of air which was passed through a series of bulbs containing concentrated sulphuric acid, in which it was supposed that whatever germs of life the air might contain would be destroyed. This flask was kept from May to August; air was passed through it daily, yet without the development of any infusorial life.

It must have been a remarkably germ-free atmosphere in which Schulze worked, for, as was shown by those who repeated his experiment, under the conditions that he regarded as certainly excluding all life, germs can readily enter with the air.

The term "infusorial life" having been used, it is well to remark that during all the early part of their recognized existence the bacteria were regarded as animal organisms and classed among the infusoria.

Tyndall, stimulated by the work of Pasteur, conclusively proved that the micro-organismal germs were in the dust suspended in the atmosphere, and not ubiquitous in distribution. His experiments were very ingenious and are of much interest. First preparing light wooden chambers, with a large glass window in the front and a smaller window in each side, he arranged a series of test-tubes in the bottom, half in and half out of the chamber, and a pipet, working through a rubber diaphragm, in the top, so that when desired the tubes, one by one, could be filled through it. Such chambers were allowed to stand until all the contained dust had settled, and then submitted to an optical test to determine the purity of the contained atmosphere by passing a powerful ray of light through the side windows. When viewed through the front, this ray was vis-

ible only so long as there were particles suspended in the atmosphere to reflect it. When the dust had completely settled and the light ray had become invisible because of the purity of the contained atmosphere, the tubes were cautiously filled with urine, beef-broth, and a variety of animal and vegetable broths, great care being taken that in the manipulation the pipet should not disturb the dust. Their contents were then boiled by submergence in a pan of hot brine placed beneath the chamber, in contact with the projecting ends of the tubes, and subsequently allowed to remain undisturbed for days, weeks, or months. In nearly every case life failed to develop in the infusions after the purity of the atmosphere was established.

The following extracts from Tyndall's work * will illustrate how slowly the doctrine of spontaneous generation was abandoned:

"At a meeting of the Pathological Society of London, held April 6, 1875, the 'germ theory' of disease was formally introduced as a subject for discussion, the debate being continued with great ability and earnestness at subsequent meetings. The conference was attended by many distinguished medical men, some of whom were profoundly influenced by the arguments, and none of whom disputed the facts brought forward against the theory on that occasion.

"The leader of the debate, and the most prominent speaker, was Dr. Bastian, to whom also fell the task of replying on all the questions raised.

"The coexistence of bacteria and contagious disease was admitted; but, instead of considering these organisms as probably the essence, or an inseparable part of the essence, of the contagium, Dr. Bastian contended that *they were pathological products spontaneously generated in the body after it had been rendered diseased by the real contagium.*

"The grouping of the ultimate particles of matter to form living organisms Dr. Bastian considered to be an operation as little requiring the action of antecedent life as their grouping to form any of the less complex chemical compounds." "Such a position must, of course, stand or fall by the evidence which its supporter is able to produce, and accordingly Dr. Bastian appeals to the law and testimony of experiment as demonstrating the soundness

* *Op. cit.*

of his view." "He seems quite aware of the gravity of the matter at hand; this is his deliberate and almost solemn appeal: 'With the view of settling these questions, therefore, we may carefully prepare an infusion from some animal tissue, be it muscle, kidney, or liver; we may place it in a flask whose neck is drawn out and narrowed in the blowpipe flame; we may boil the fluid, seal the vessel during ebullition, and, keeping it in a warm place, may await the result, as I have often done. . . . After a variable time the previously heated fluid within the hermetically sealed flasks swarms more or less plentifully with bacteria and allied organisms, even though the fluids have been much degraded in quality by exposure to the temperature of 212° F., and have in all probability been rendered far less prone to engender independent living units than the unheated fluids in the tissues would be.'"

II. CHEMIC CONTRIBUTIONS; FERMENTATION AND PUTREFACTION.

As in the world of biology the generation of life was an all-absorbing problem, so in the world of chemistry the phenomena of fermentation and putrefaction were inexplicable so long as the nature of the ferments was not understood.

In the year 1837 Latour and Schwann succeeded in demonstrating that the minute oval bodies which had been observed in yeast since the time of Leeuwenhoek were living organisms—vegetable forms—capable of growth.

So long as yeast was looked upon as an inert substance it was impossible to understand how it could impart fermentation to other substances; but when it was shown by Latour that the essential element of yeast was a growing plant, the phenomenon became a perfectly natural consequence of life. Not only the alcoholic, but also the acetic, lactic, and butyric fermentations have been shown to result from the energy of low forms of vegetable life, chiefly bacterial in nature. Prejudice, however, prevented many chemists from accepting this view of the subject, and Liebig strenuously adhered to his theory that fermentation was the result of the internal molecular movements which a body in the course of decomposition communicates to other matter whose elements are connected by a very feeble affinity.

Pasteur was the first to prove that fermentation is an

ordinary chemic transformation of certain substances, taking place as the result of the action of living cells, and that the capacity to produce it resides in all animal and vegetable cells, though in varying degree.

In 1862 he published a paper "On the Organized Corpuscles Existing in the Atmosphere," in which he showed that many of the floating particles collected from the atmosphere of his laboratory were organized bodies. If these were planted in sterile infusions, abundant crops of micro-organisms were obtained. By the use of more refined methods he repeated the experiments of others, and showed clearly that "the cause which communicated life to his infusions came from the air, but was not evenly distributed through it."

Three years later he showed that the organized corpuscles which he had found in the air were the spores or seeds of minute plants, and that many of them possessed the property of withstanding the temperature of boiling water—a property which explained the peculiar results of many previous experimenters, who failed to prevent the development of life in boiled liquids inclosed in hermetically sealed flasks.

Chevreul and Pasteur, by having proved that animal solids do not putrefy or decompose if kept free from the access of germs, suggested to surgeons that putrefaction in wounds is due rather to the entrance of something from without than to changes within. The deadly nature of the discharges from putrescent wounds had been shown in a rough manner by Gaspard as early as 1822 by injecting some of the material into the veins of animals.

III. MEDICAL AND SURGICAL CONTRIBUTIONS; THE STUDY OF THE INFECTIOUS DISEASES.

Probably the first writing in which a direct relationship between micro-organisms and disease is suggested is by Varro, who says: "It is also to be noticed, if there be any marshy places, that certain minute animals breed [there] which are invisible to the eye, and yet, getting into the system through mouth and nostrils, cause serious disorders (diseases which are difficult to treat)."

Surgical methods of treatment depending for their success upon exclusion of the air, and of course, incidentally if unknowingly, exclusion of bacteria, seem to have been

practised quite early. Theodoric, of Bologne, about 1260 taught that the action of the air upon wounds induced a pathologic condition predisposing to suppuration. He also treated wounds with hot wine fomentations. The wine was feebly antiseptic, kept the surface free from bacteria, and the treatment was, in consequence, a modification of what in later centuries formed antiseptic surgery.

Henri de Mondeville in 1306 went even further than Theodoric, whom he followed, and taught the necessity of bringing the edges of a wound together, covered it with an exclusive plaster compounded of turpentine, resin, and wax, and then applied the hot wine fomentation.

In 1671 Kircher wrote a book in which he expressed the opinion that puerperal fever, purpura, measles, and various other fevers were the result of a putrefaction caused by worms or animalcules. His opinions were thought by his contemporaries to be founded upon too little evidence, and were not received.

Plencig, of Vienna, became convinced that there was an undoubted connection between the microscopic animalcules exhibited by the microscope and the origin of disease, and advanced this opinion as early as 1762. Unfortunately, the opinions of Plencig seem not to have been accepted by others, and were soon forgotten.

In 1704 John Colbach described "a new and secret method of treating wounds by which healing took place quickly, without inflammation or suppuration."

Boehm succeeded in 1838 in demonstrating the occurrence of yeast plants in the stools of cholera, and conjectured that the process of fermentation was concerned in the causation of that disease.

In 1840 Henle considered all the evidence that had been collected, and concluded that the cause of the infectious diseases was to be sought for in minute living organisms or fungi. He may be looked upon as the real propounder of the Germ Theory of Disease, for he not only collected facts and expressed opinions, but also investigated the subject ably. The requirements which he formulated in order that the theory might be proved were so severe that he was never able to attain to them with the crude methods at his disposal. They were so ably elaborated, however, that in after years they were again postulated by Koch, and it is only by strict conformity with them

that the definite relationship between bacteria and disease has been determined.

Briefly summarized, these requirements are as follows:

1. A specific micro-organism must be constantly associated with the disease.

2. It must be isolated and studied apart from the disease.

3. When introduced into healthy animals it must produce the disease, and in the animal in which the disease has been experimentally produced the organism must be found under the original conditions.

In 1849 J. K. Mitchell, in a brief work upon the "Cryptogamous Origin of Malarious and Epidemic Fevers," foreshadowed the germ theory of disease by collecting a large amount of evidence to show that malarial fevers were due to infection by fungi.

Pollender (1849) and Davaine (1850) succeeded in demonstrating the presence of the anthrax bacillus in the blood of animals suffering from and dead of that disease. Several years later (1863) Davaine, having made numerous inoculation experiments, demonstrated that this bacillus was the *materies morbi* of the disease. The bacillus of anthrax was probably the first bacterium shown to be specific for a disease. Being a very large bacillus and a strongly vegetative organism, its growth was easily observed, while the disease was one readily communicated to animals.

In 1873 Obermeier observed that actively motile, flexible spiral organisms were present in large numbers in the blood of patients in the febrile stages of relapsing fever.

Klebs, who was one of the pioneers of the germ theory, published, in 1872, a work upon septicemia and pyemia, in which he expressed himself convinced that the causes of these diseases must come from without the body. Billroth, however, strongly opposed such an idea, asserting that fungi had no especial importance either in the processes of disease or in those of decomposition, but that, existing everywhere in the air, they rapidly developed in the body as soon as through putrefaction a "Faulnisszymoid" (putrefactive ferment), or through inflammation a "Phlogistischezymoid" (inflammatory ferment), supplying the necessary feeding-grounds, was produced.

In 1875 the number of scientific men who had entirely abandoned the doctrine of spontaneous generation and embraced the germ theory of disease was small, and most of those who accepted it were experimenters. A great

majority of medical men either believed, like Billroth, that the presence of fungi where decomposition was in progress was an accidental result of their universal distribution, or, being still more conservative, adhered to the old notion that the bacteria, whose presence in putrescent wounds as well as in artificially prepared media was unquestionable, were spontaneously generated there.

Before many of the important bacteria had been discovered, and while ideas upon the relation of micro-organisms to disease were most crude, some practical measures were suggested that produced greater agitation and incited more observation and experimentation than anything suggested in surgery since the introduction of anesthetics—namely, *antisepsis*.

"It is to one of old Scotia's sons, Sir Joseph Lister, that the everlasting gratitude of the world is due for the knowledge we possess in regard to the relation existing between micro-organisms and inflammation and suppuration, and the power to render wounds aseptic through the action of germicidal substances." *

Lister, convinced that inflammation and suppuration were due to the entrance of germs from the air, instruments, fingers, etc., into wounds, suggested the employment of carbolic acid for the purpose of keeping sterile the hands of the operator, the skin of the patient, the surface of the wound, and the instruments used. He finally concluded every operation by a protective dressing to exclude the entrance of germs at a subsequent period.

Listerism, or "antisepsis," originated in 1875, and when Koch published his famous work on the "Wundinfectionskrankheiten" (traumatic infectious diseases), in 1878, it spread slowly at first, but surely in the end, to all departments of surgery and obstetrics.

From time to time, as the need for them was realized, the genius of investigators provided new devices which materially aided in their work, and have made possible many discoveries that must otherwise have failed. Among them may be mentioned the improvement of the compound microscope, the use of sterilized culture fluids by Pasteur, the introduction of solid culture media and the isolation methods by Koch, the use of the cotton plug by Schroeder and van Dusch, and the introduction of the anilin dyes by Weigert.

It is interesting to note that after the discovery of the

* Agnew's "Surgery," vol. I, chap. II.

anthrax bacillus by Pollender and Davaine, in 1849, there was a period of nearly twenty-five years during which no important pathogenic organisms were discovered, but during which technical methods were being elaborated, making possible a rapid succession of subsequent important discoveries.

Thus, in 1873, Obermeier discovered Spirillum obermeieri of relapsing fever.

In 1879 Hansen announced the discovery of bacilli in the cells of leprous nodules, and Neisser discovered the gonococcus.

In 1880 the bacillus of typhoid fever was observed by Eberth and independently by Koch, Pasteur published his work upon "Chicken-cholera," and Sternberg described the pneumococcus, calling it *Micrococcus pasteuri.*

In 1882 Koch made himself immortal by his discovery of and work upon the tubercle bacillus, and in the same year Pasteur published a work upon "Rouget du Porc," and Löffler and Shütz discovered the bacillus of glanders.

In 1884 Koch reported the discovery of the "comma bacillus," the cause of cholera, and in the same year Löffler isolated the diphtheria bacillus, and Nicolaier the tetanus bacillus.

In 1892 Canon and Pfeiffer discovered the bacillus of influenza.

In 1894 Yersin and Kitasato independently isolated the bacillus causing the bubonic plague then prevalent at Hong-kong.

A new era in bacteriology, and probably the most triumphant achievement of scientific medicine, was inaugurated when, in 1890, Behring explained to the world the principles of the "blood-serum therapy," and since that time the chief investigations have been along the lines of immunity, immunization and the therapeutic serums, the names of Behring, Kitasato, Wernicke, Roux, Ehrlich, Metschnikoff, Bordet, Wassermann, Shiga, Madsen, and Arrhenius, taking front rank.

CHAPTER I.

BACTERIA.

Definition.—Bacteria (from the Greek *βακτήριον*, a rod) are minute unicellular organisms, now generally conceded to belong to the vegetable kingdom and classed among the lowest thallophytes.

Classification of Bacteria.—The bacteria were thought by Leeuwenhoek and his successors, even to the time of Ehrenberg and Dujardin, to be animalcules belonging to the infusoria, and were in consequence placed in the animal kingdom. With our present knowledge they can unhesitatingly be placed in the vegetable kingdom, among the lower orders of the flowerless plants. The flowerless plants or *Cryptogamia* include the *Pteridophytæ;* the *Bryophytæ*, including the ferns and liverworts; and the *Thallophytæ*, including the *algæ* and *fungi*. It is among the Thallophytæ, and probably among the fungi, that bacteria belong.

The algæ differ from the fungi in possessing chlorophyl, and as the bacteria sometimes contain chlorophyl, it becomes a question whether they should be included in either of these groups or form a group by themselves; and their classification among the fungi is held by botanists to be provisional.

The scientific grouping of the bacteria themselves has not yet been achieved, the best characters to be used as the basis of classification being undecided. The best system for their provisional arrangement is probably that of Migula,* or the modification of it suggested by F. D. Chester,† in which the morphology, sporulation, and appendages of the bacteria all enter as important features.

* "System der Bakterien," Jena, 1897–1900 (vols. I and II appearing at different times).

† "Preliminary Arrangement of the Species of the Genus Bacterium," "Ninth Annual Report of the Delaware College Agricultural Experiment Station," 1897; Newark, Delaware, U. S. A.

BACTERIA.

1. Family Coccaceæ. Cells globular, becoming slightly elongate before division. Division in one, two, or three directions of space. Formation of endospores very rare.
   (A) Without flagella.
      1. *Streptococcus.* Division in one direction of space, producing chains like strings of beads.
      2. *Micrococcus.* Division in two directions of space, so that tetrads are often formed.
      3. *Sarcina.* Division in three directions of space, leading to the formation of bale-like packages.
   (B) With flagella.
      1. *Planococcus.* Division in two directions of space, like micrococcus.
      2. *Planosarcina.* Division in three directions, like sarcina.
2. Family Bacteriaceæ. Cells more or less elongate, cylindric, and straight. They never form spiral windings. Division in one direction of space only, transverse to the long axis of the cell.
   (A) Without flagella.
      1. *Bacterium.* Occasional endospores.
   (B) With flagella.
      2. *Bacillus.* Flagella arising from any part of the surface. Endospore-formation common.
      3. *Pseudomonas.* Flagella attached only at the ends of the cell. Endospores very rare.
3. Family Spirillaceæ. Cells twisted spirally like a corkscrew, or representing sections of the spiral. Division only transverse to the long diameter.
      1. *Spirosoma.* Rigid; without flagella.
      2. *Microspira.* Rigid; having one, two, or three undulating flagella at the ends.
      3. *Spirillum.* Rigid; having from five to twenty curved or undulating flagella at the ends.
      4. *Spirochæta.* Serpentine and flexible. Flagella not observed; probably swim by means of an undulating membrane.
4. Family Mycobacteriaceæ. Cells forming long or short cylindric filaments, often clavate-cuneate or irregular in form, and at times showing true or false branchings. No endospores, but formation of gonidia-like bodies due to segmentation of the cells. No flagella. Division at right angles to the axis of rod in filament. Filaments not surrounded by a sheath as in Chlamydobacteriaceæ.
      1. *Mycobacterium.* Cells in their ordinary form, short cylindric rods often bent and irregularly cuneate. At times Y-shaped forms or longer filaments with true branchings may produce short coccoid elements, perhaps gonidia. (This genus includes the Corynebacterium of Lehmann-Neumann.) No flagella.
      2. *Actinomyces.* Cells in their ordinary form as long branched filaments; growth coherent, dry or crumpled. Produce gonidia-like bodies. Cultures generally have a moldy appearance, due to the development of aërial hyphæ. No flagella.
5. Family Chlamydobacteriaceæ. Forms that vary in different stages of their development, but all characterized by a surrounding sheath about both branched and unbranched threads. Division transverse to the length of the filaments.

1. *Cladothrix.* Characterized by pseudo-dichotomous branchings. Division only transverse. Multiplication by the separation of whole branches. Transplantation by means of polar flagellated swarm-spores.
2. *Crenothrix.* Cells united to form unbranched threads which in the beginning divide transversely. Later the cells divide in all three directions of space. The products of final division become spheric, and serve as reproductive elements.
3. *Phragmidiothrix.* Cells at first united into unbranched threads. Divide in three directions of space. Late in the development, by the growth of certain of the cells through the delicate, closely approximated sheath, branched forms are produced.
4. *Thiothrix.* Unbranched cells inclosed in a delicate sheath. Non-motile. Division in one direction of space. Cells contain sulphur grains.

6. Family BEGGIATOACEÆ. Cells united to form threads which are not surrounded by an inclosing sheath. The septa are scarcely visible. Divide in one direction of space only. Motility accomplished through the presence of an undulating membrane.
1. *Beggiatoa.* Cells contain sulphur grains.

Structure.—*Nucleus.*—When subjected to the action of nuclear stains, vague formations are sometimes observed in the interior of the bacterial cells.

Cytoplasm.—The cytoplasm, of which very little exists between the large nucleus and cell wall, is sometimes granular, as in Bacillus megatherium, and sometimes contains fine granules of chlorophyl, sulphur, fat, or pigment.

Capsule.—Each cell is surrounded by a distinct cell-wall, which in some species shows the cellulose reaction with iodin.

The cell-walls of certain bacteria seem at times to undergo a peculiar gelatinous change or to permit the exudation of gelatinous material from the cytoplasm, and appear surrounded by a halo or capsule. Such capsules are seen about the pneumococcus as found in blood or sputum, Friedländer's bacillus as seen in sputum, Bacillus aerogenes capsulatus in blood or tissue, and many other organisms. Friedländer points out that the capsule of his pneumonia bacillus, as found in the lung tissue or in the "prune-juice" sputum, was very distinct, though it could not be demonstrated at all when the organisms grew in gelatin.

The anilin dyes, which possess a great penetrating power, color the organisms so intensely that they usually appear as uniformly colored spheres, rods, or spirals.

Polar Granules.—By carefully staining an appropriate organism certain peculiarities of structure can sometimes be shown. Thus, some bacilli contain distinct "polar gran-

ules" (metachromatic or Babes-Ernst granules)—rounded or oval unstained bodies—observed at the ends of the cell. Their significance is unknown. They have been supposed to bear some relationship to the biologic activity of the organism, especially its pathogenesis, but this is uncertain, and a recent work by Gauss* and Schumburg† shows that they vary with the reaction of the culture media upon which the bacteria grow and have nothing to do with their virulence. Bacillus megatherium is peculiar in having its cytoplasm filled with small granules which stain deeply. The diphtheria bacillus and the cholera spirillum stain very irregularly in fresh cultures, as if the tingeable substance were not uniformly distributed throughout the cytoplasm. Vacuolated bacteria and bacteria that will not stain, or stain very irregularly, may usually be regarded as degenerated organisms (involution forms) which, because of *plasmolysis*, or solution, can no longer stain homogeneously.

Flagella.—Many bacteria possess delicate straight or wavy filaments called *cilia* or *flagella*, which appear to be organs of locomotion. Sometimes they are attached only at the ends, sometimes to all parts of the organisms.

Messea‡ has suggested that the bacteria be classified, according to the arrangement of the flagella, into:

I. Gymnobacteria (forms without flagella).
II. Trichobacteria (forms with flagella).
    1. Monotricha (with a single flagellum at one end).
    2. Lophotricha (with a bundle of flagella at one end).
    3. Amphitricha (with a flagellum at each end).
    4. Peritricha (flagella around the body, springing from all parts of its surface).

This arrangement is, however, less satisfactory than that of Migula already given.

Motility.—The greater number of the bacteria supplied with flagella are actively motile, the locomotory power no doubt being the lashing flagella. The rod and spiral microorganisms are most plentifully supplied with flagella; only a few of the spheric forms have them.

The presence of flagella, however, does not invariably

* "Centralbl. f. Bakt.," etc., XXXI, No. 3, Feb. 5, 1902, p. 106.
† *Ibid.*, XXXI, No. 14, p. 694, June 3, 1902.
‡ "Rivista d'igiene e sanata publica," 1890, II.

imply motility, as they may also serve to stimulate the passage of currents of nutrient fluid past the organism, and so favor its nutrition. The flagellate bacteria are more numerous among the saprophytic bacteria found in water or other fluids, than among the pathogenic forms found in the tissues, though it may be added that parasitic bacteria not habitually entering the tissues, but inhabiting the intestine, as the bacillus of typhoid fever and the spirillum of cholera, are actively motile, like the saprophytes.

Bacillus megatherium has a distinct but limited ameboid movement.

The dancing movement of some of the spheric bacteria seems to be the well-known Brownian movement, which is a physical phenomenon. It is sometimes difficult to determine whether an organism viewed under the microscope is really motile or whether it is only vibrating. One can usually determine by observing that in the latter case it does not change its relative position to surrounding objects.

In some cases the colonies of actively motile bacteria, such as the proteus bacilli, show definite migratory tendencies upon 5 per cent. gelatin. The active movement of the bacteria composing the colony causes its shape constantly to change, so that it bears a faint resemblance to an ameba, and moves about from place to place upon the surface of the gelatin.

Size.—Bacteria are so minute that a special unit has been adopted for their measurement. This is the *micromillimeter* (μ), or one-thousandth part of a millimeter, equivalent to the one-twenty-five-thousandth ($\frac{1}{25000}$) of an inch.

The size of bacteria varies from a fraction of a micromillimeter to 20 or even 40 micromillimeters.

Reproduction.—Fission.—Bacteria multiply by binary division (fission). A bacterium about to divide appears larger than normal, and, if a spheric organism, more or less ovoid. By appropriate staining karyokinetic changes may be observed in the nuclei. When the conditions of nutrition are good, fission progresses with astonishing rapidity. Buchner and others have determined the length of a generation to be from fifteen to forty minutes.

The results of binary division, if rapidly repeated, are almost appalling. "Cohn calculated that a single germ could produce by simple fission two of its kind in an hour; in the second hour these would be multiplied to four;

and in three days they would, if their surroundings were ideally favorable, form a mass which can scarcely be reckoned in numbers." "Fortunately for us," says Woodhead, "they can seldom get food enough to carry on this appalling rate of development, and a great number die both for want of food and because of the presence of other conditions unfavorable to their existence."

Sporulation.—When the conditions for rapid multiplication by fission are no longer good, many of the organisms guard against extinction by developing small eggs, seeds, or, as they are correctly called, *spores* (Fig. 1).

Endospores.—Spores developed within the bacteria are called *endospores.*

Endospores are generally formed in the elongate bacteria, —bacillus and spirillum,—but Zopf has observed similar bodies in micrococci. Escherich also claims to have found undoubted spores in a sarcina.

Spores may be either round or oval. As a rule, each

Fig. 1.—Diagram illustrating sporulation: *a*, Bacillus inclosing a small oval spore; *b*, drumstick bacillus, with the spore at the end; *c*, clostridium; *d*, free spores; *e* and *f*, bacilli escaping from spores.

organism produces a single spore, which is situated either at its center or at its end. When, as sometimes happens, the diameter of the spore is greater than that of the bacillus, it causes a peculiar barrel shape bulging of the organism, described as *clostridium.* When the distending spore is at the end, a "Trommelschläger," or "drumstick," is formed. End-spores are almost characteristic of anaerobic bacilli. When the formation of a spore is about to commence, a small bright point appears in the cytoplasm, and increases in size until its diameter is nearly or quite as great as that of the bacterium. A dark, highly refracting capsule is finally formed about it. As soon as the spore arrives at perfection the bacterium seems to die, as if its vitality were exhausted in the development of the permanent form. As the degeneration of the cytoplasm sets the spore free, it appears as a clear, highly refracting sphere or ovoid.

The spores differ from the bacteria in that their capsules prevent evaporation and enable them to withstand drying and the application of a considerable degree of heat. Very few adult bacteria are able to resist temperatures above 70° C. Spores are, however, uninjured by such temperatures, and can even successfully resist the temperature of boiling water (100° C.) for a short time. The extreme desiccation caused by a protracted exposure to a dry temperature of 150° C. will invariably destroy them, as will also steam under pressure. Not only can the spores successfully resist a considerable degree of heat, but they are also unaffected by cold of almost any intensity, and the duration of their vitality is very remarkable. Von Szekely* found anthrax spores capable of germination after eighteen years and six months in some dried-up old gelatin cultures found in his laboratory.

Arthrospores.—The formation of *arthrospores* is less clear, and seems to be the conversion of the entire organism into a spore or permanent form. Arthrospores have been observed particularly among the micrococci, where certain individuals become enlarged beyond the normal, and surrounded by a capsule. Hüppe, who has paid particular attention to the arthrospores, believes that they have resisting powers far greater than those possessed by the bacteria themselves. Of the arthrospores little has, so far, been learned. It is not improbable that among the micrococci, and also among some of the smaller bacilli in which no spores have been observed, the maintenance of the species when conditions of life become unfavorable is due to the assumption of a permanent form by some of the individuals, without the formation of any spore-like bodies.

Though the cell-wall of the adult bacterium is easily penetrated by solutions of the anilin dyes, it is difficult to stain spores, which are distinctly more resistant to the action of chemic agents than the bacteria themselves.

Germination of Spores.—When a spore is about to germinate, the contents, which have been clear and transparent, become granular, the body increases slightly in size, the capsule becomes less distinct, and in the course of time splits open to allow the escape of a young organism. The direction in which the capsule ruptures varies in different species. Bacillus subtilis escapes from the side of the spore; Bacillus anthracis from the end. This difference can be

3 * "Zeitschr. für Hygiene," 1903, XLIV, 3.

made use of as an aid in differentiating otherwise similar organisms.

So soon as the young bacillus escapes it begins to increase in size, develops a characteristic capsule, and presently begins the propagation of its species by fission.

It is believed by Fränkel and others that sporulation is not a sign that the food-supply has failed, but a sign that the vital perfection of the organism has been attained. These observers regard spore-formation in the bacteria as analogous to the flowering of higher plants, which takes place only when the conditions of development are best.

Morphology.—Three principal forms of bacteria exist, from which all others seem to be but variations. They are spheres (cocci), rods (bacilli), and screws (spirilla).

Cocci.—The spheric bacteria, from a fancied resemblance to little berries, are called *cocci* or *micrococci* (Fig. 2, *a*). According to peculiarities of multiplication, they are subject to certain subdivisions. When cocci divide, and the resulting organisms remain attached to one another, a *diplococcus* (Fig. 2, *b*) is produced. Diplococci may consist of two at-

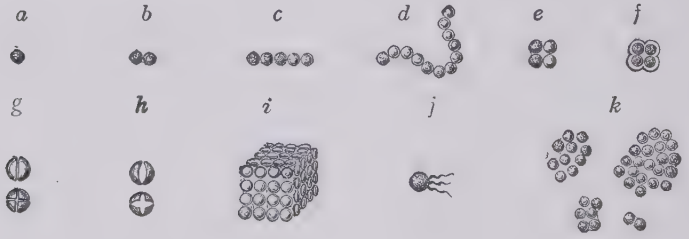


Fig. 2.—Diagram illustrating the morphology of the cocci: *a*, Coccus or micrococcus; *b*, diplococcus; *c*, *d*, streptococci; *e*, *f*, tetracocci or merismopedia; *g*, *h*, modes of division of cocci; *i*, sarcina; *j*, coccus with flagella; *k*, staphylococci.

tached spheres, though each half commonly shows flattening of the contiguous surfaces (Fig. 2, *g*). In a few cases, as the gonococcus, the approximated surfaces may be slightly concave, causing the organism to resemble the German biscuit called a "Semmel" (Fig. 2, *h*). When a second binary division occurs, and four resulting individuals remain attached to one another, without disturbing the arrangement of the first two, a tetrad, or *tetracoccus*, is formed. To the entire groups of cocci dividing in two

directions of space so as to produce fours, eights, twelves, etc., on the same plane, the name *merismopedia* has been given (Fig. 2, *e* and *f*). Migula uses the term *micrococcus* for the unflagellated tetrads, and *planococcus* for the flagellated forms.

If division take place in three directions of space, so as to produce a cubic "package" of cocci, the resulting aggregation is described as a *sarcina* (Fig. 2, *i*). This form resembles a dice or a miniature bale of cotton. Few sarcinæ have flagella, similar flagellated organisms being called by Migula *planosarcina*.

If division always take place in the same direction, so that the cocci remain attached to one another like a string of beads, the organism is described as a *streptococcus* (Fig. 2, *d*).

Cocci commonly occur in irregular groups having a fancied resemblance to bunches of grapes. Such are called *staphylococci*, and most organisms not finding a place in the varieties already described are so classed.

Cocci associated in globular or lobulated clusters incased in a resisting gelatinous, homogeneous mass, have been described by Billroth as *ascococcus*. Cocci, solitary or in

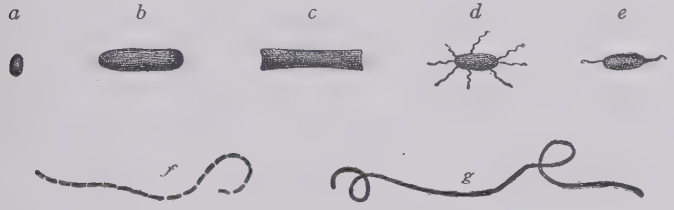


Fig. 3.—Diagram illustrating the morphology of the bacilli: *a*, *b*, *c*, Various forms of bacilli; *d*, *e*, bacilli with flagella; *f*, chain of bacilli, individuals distinct; *g*, chain of bacilli, individuals not separated.

chains, surrounded by an incasement of almost cartilaginous consistence, have been called *leuconostoc*.

Bacilli.—A better known, if not more important, group of bacteria are elongate or "rod-shaped," and bear the name *bacillus* (a rod) (Fig. 3).

The bacilli present considerable variation of form. Some are elliptoid, some long and slender. Some have rounded ends, as B. subtilis; others have square ends, as B. anthracis. Some are large, some exceedingly small. Some always occur singly, never uniting to form threads or chains; others are nearly always so conjoined.

The bacilli divide by transverse fission only, so that the only peculiarity of arrangement is the formation of threads or chains.

Bacterium.—In the older writings, short, stout bacilli were described under the generic term *bacterium*. Migula now employs the term to include only bacillary forms without flagella.

Pseudomonas.—A *pseudomonas* is a bacillary form with polar flagella.

Vibrio.—Some of the flexile bacilli have sinuous movements resembling the swimming of a snake or an eel, and are sometimes described as *vibrio;* but this name also has passed into disuse, except in France, where spiral organisms are so called.

Long filaments formed by division of bacilli without distinct separations are sometimes called *leptothrix*, and when such threads form tangled masses surrounded by a jelly-like material, the name *myconostoc* is sometimes applied to them.

Spirilla.—If a rod-shaped bacterium is spirally twisted

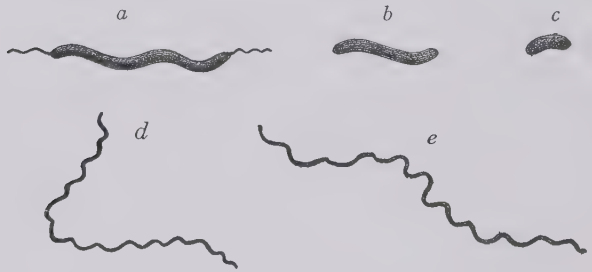


Fig. 4.—Diagram illustrating the morphology of the spirilla: *a*, *b*, *c*, Spirilla; *d*, *e*, spirochæta.

and resembles a corkscrew, it is called *spirillum* (Fig. 4). The rigid forms without flagella are known as *spirosoma;* rigid forms with flagella, *spirilla* and *microspira*. The flexile forms are called *spirochæta* and probably have no flagella.

A spiral organism of ribbon shape is called *spiromonas*, while a similar organism of spindle shape is called a *spirulina*. One species of spiral bacteria in whose cytoplasm sulphur granules have been detected has been called *ophidiomonas*.

Some of the spirilla are long and slender; others, like the spirillum of cholera, are so short as to be easily mistaken for slightly bent bacilli.

Since the studies of Schaudinn* and others upon the trypanosoma, great care must be exercised in the study of the spiral organisms, lest sexual or developmental stages of higher animal organisms be mistaken for spirilla or spirochæta.

THE HIGHER BACTERIA.

The Higher Bacteria are characterized by filamentous forms with real or apparent branchings. The filaments are usually regularly divided transversely, so as to appear as if composed of bacilli. The free ends only seem to be endowed with reproductive functions, and develop peculiar bodies known as *conidia*. These organisms resemble the oidia and molds in many particulars.

It is the specialization of certain elements for purposes of vegetation, reproduction, etc., that differentiates the higher from the other bacteria whose cells are all free and equally independent.

* "Mitt. aus dem Kaiserl. Gesundheitsamte."

CHAPTER II.

BIOLOGY OF BACTERIA.

The distribution of bacteria is well-nigh universal. They and their spores float in the atmosphere we breathe, swim in the water we drink, grow upon the food we eat, and luxuriate in the soil beneath our feet. Nor is this all, for, entering the palpebral fissures, they develop upon the conjunctiva; entering the nares, they establish themselves in the nose; the mouth is always replete with them; and, as many are swallowed, the digestive apparatus always contains them. The surface of the body never escapes them, and so deeply are some individuals situated among the epithelial cells of the skin that the most careful washing and scrubbing and the use of the most powerful germicides are required to rid the surgeon's hands of what may prove to be dangerous hindrances to the healing of wounds. The ear is not without its microscopic flora; special varieties live beneath the finger-nails, and especially the toe-nails, in the vagina, and beneath the prepuce.

They are not, however, ubiquitous, and Tyndall* succeeded in proving that the atmosphere of high Alpine altitudes was free from them, and likewise that the glacier ice contained none; but wherever man, animals, or even plants, live, die, and decompose, bacteria are sure to be present.

Notwithstanding their intimate relationship with the animal body, the *body-juices and tissues of normal animals are free from them, and their occasional occurrence there may be accepted as a sign of disease.*

The presence of bacteria in the air generally depends upon their previous existence in the soil, its pulverization and distribution by currents of the atmosphere. Koch has shown that the upper stratum of the soil is exceedingly rich in bacteria, but that their numbers decrease as the soil is penetrated, until below a depth of one meter there are very few. Remembering that bacteria live chiefly upon

* "Floating Matter in the Air."

organic matter, this is readily understandable, as most of the organic matter is upon the surface of the soil. Where, as in the case of porous soil or the presence of cesspools and dung-heaps, the decomposing materials are allowed to penetrate to a considerable depth, the bacteria may occur much farther below the surface; yet they are rarely found at any great depth, because the majority of them require free oxygen for successful existence.

The water of stagnant pools always teems with bacteria, but that of deep wells rarely contains many unless it is polluted from the surface of the earth.

Being generally present in the soil, which the feet of men and animals grind to powder, the bacteria, together with the pulverized earth, are blown from place to place into every nook and cranny, until it is impossible to escape them. It has been suggested by Soyka that currents of air passing over the surface of liquids might take up bacteria, but, although he seemed to show it experimentally, it is not generally believed. Where bacteria are growing in colonies they seem to remain undisturbed by currents of air unless the surface of the colony becomes roughened or broken.

Most of the bacteria that are carried about by the air are what are called saprophytes, and are perfectly harmless to the human being; but not all belong to this class, nor will they do so while tuberculous patients are allowed to expectorate upon the sidewalks, and typhoid patients' wash to dry upon the clothes-line, and their dejecta to be spread upon the ground.

The growth of bacteria is profoundly influenced by environment, so that a consideration of the conditions favorable and unfavorable to their existence becomes necessary.

Conditions Favoring the Growth of Bacteria.—(a) **Oxygen.**—As all micro-organisms must have oxygen in order to live, the greater number of them grow best when freely exposed to the air. Some will not grow at all where uncombined oxygen is present, but secure all they need by severing it from its chemic combinations These peculiarities divide bacteria into the

Aerobes, which grow in the presence of uncombined oxygen, and the

Anaerobes, which do not grow in the presence of uncombined oxygen.

As, however, some of the aerobic forms grow almost as well without oxygen as with it, they are known as *optional* (facultative) *anaerobes*.

As examples of strictly aerobic bacteria Bacillus subtilis, Bacillus aerophilus, Bacillus tuberculosis, and Bacillus diphtheriæ may be given. These will not grow if oxygen is denied them. The cocci of suppuration, the bacillus of typhoid fever, and the spirillum of cholera grow almost equally well with or without oxygen, and hence belong to the optional anaerobes. The bacilli of tetanus and of malignant edema, and the non-pathogenic Bacillus butyricus, Bacillus muscoides, and Bacillus polypiformis, will not develop at all where any free oxygen is present, and hence are strictly anaerobic.

(b) **Nutriment.**—The bacteria grow best where diffusible albumins are present, the ammonium salts being less fitted to support them than their organic compounds. Proskauer and Beck* have succeeded in growing the tubercle bacillus in a mixture containing ammonium carbonate 0.35 per cent., potassium phosphate 0.15 per cent., magnesium sulphate 0.25 per cent., and glycerin 1.5 per cent. Some of the water microbes can live in distilled water to which the smallest amount of organic matter has been added; others require so concentrated a medium that only blood-serum can be used for their cultivation. The statement that certain forms of bacteria can flourish in clean distilled water seems to be untrue, as in this medium the organisms soon die and disintegrate. If, however, in making the transfer, a drop of culture material is carried into the water with the bacteria, the distilled water ceases to be such, and becomes a dilute bouillon fitted to support bacterial life for a time. Sometimes a species with a preference for a particular culture medium can gradually be accustomed to another, though immediate transplantation causes the death of the organism. Sometimes the addition of such substances as glucose and glycerin has a peculiarly favorable influence, the latter, for example, enabling the tubercle bacillus to grow upon agar-agar.

(c) **Moisture.**—A certain amount of water is indispensable to the growth of bacteria. The amount can be

* "Zeitschrift für Hygiene," etc., Aug. 10, 1894, vol. xviii, No. 1.

exceedingly small, however, Bacillus prodigiosus being able to develop successfully upon crackers and dried bread. Artificial culture media should not be too concentrated; at least 80 per cent. of water should be present.

(d) **Reaction.**—Should the pabulum supplied to bacteria contain an excess of either alkali or acid, the growth of the organisms is inhibited. Most true bacteria grow best in a neutral or feebly alkaline medium. There are exceptions to this rule, however, for Bacillus butyricus and Sarcina ventriculi can grow well in strong acids, and Micrococcus urea can tolerate excessive alkalinity. Acid media are excellent for the cultivation of molds.

Conditions Prejudicial to the Growth of Bacteria.—(a) **Light.**—Most bacteria are not influenced by the presence or absence of ordinary diffused daylight. The direct rays of the sun, and to a less degree the rays of the electric arc-light, retard and in numerous instances kill bacteria. Certain colors are distinctly inhibitory to their growth, blue being especially prejudicial. Some of the chromogenic forms produce their colors only when exposed to the ordinary light of the room. Bacillus mycoides roseus produces its red pigment only in the dark. The virulence of many pathogenic bacteria is gradually attenuated if they are kept in the light.

(b) **Electricity, X-rays, etc.**—Very powerful currents of electricity passed through cultures of bacteria have been found to kill the organisms and change the reaction of the culture medium; rapidly reversed currents of high intensity to destroy the pathogenesis of the bacteria and transform their toxic products into neutralizing protective bodies (antitoxin?). Attention has been called to this subject by Smirnow, d'Arsonval and Charin, Bolton and Pease, Bonome and Viola, and others.

The most thorough and important contribution upon the "Effect of Direct, Alternating, Tesla Currents and X-rays on Bacteria" is by Zeit.* The technical methods adopted make it worth while for the student to refer to the original paper. The conclusions to which the author comes are as follows:

1. A continuous current of 260 to 320 milliamperes passed through bouillon cultures kills bacteria of low thermal death-points in ten minutes by the production of heat

* "Jour. Amer. Med. Assoc.," Nov. 30, 1901.

98.5° C. The antiseptics produced by electrolysis during this time are not sufficient to prevent the growth of even non-spore-bearing bacteria. The effect is a purely physical one.

2. A continuous current of 48 milliamperes passed through bouillon cultures for from two to three hours does not kill even non-resistant forms of bacteria. The temperature produced by such a current does not rise above 37° C., and the electrolytic products are antiseptic, but not germicidal.

3. A continuous current of 100 milliamperes passed through bouillon cultures for seventy-five minutes kills all non-resistant forms of bacteria even if the temperature is artificially kept below 37° C. The effect is due to the formation of germicidal electrolytic products in the culture. Anthrax spores are killed in two hours. Subtilis spores were still alive after the current was passed for three hours.

4. A continuous current passed through bouillon cultures of bacteria produces a strongly acid reaction at the positive pole, due to the liberation of chlorin which combines with oxygen to form hypochlorous acid. The strongly alkaline reaction of the bouillon culture at the negative pole is due to the formation of sodium hydroxid and the liberation of hydrogen in gas bubbles. With a current of 100 milliamperes for two hours it required 8.82 milligrams of H_2SO_4 to neutralize 1 c.c. of the culture fluid at the negative pole, and all the most resistant forms of bacteria were destroyed at the positive pole, including anthrax and subtilis spores. At the negative pole anthrax spores were killed also, but subtilis spores remained alive for four hours.

5. The continuous current alone, by means of Du Bois-Reymond's method of non-polarizing electrodes, and exclusion of chemic effects by ions in Kruger's sense, is neither bactericidal nor antiseptic. The apparent antiseptic effect on suspension of bacteria is due to electric osmose. The continuous electric current has no bactericidal nor antiseptic properties, but can destroy bacteria only by its physical effects (heat) or chemic effects (the production of bactericidal substances by electrolysis).

6. A magnetic field, either within a helix of wire or between the poles of a powerful electro-magnet, has no antiseptic or bactericidal effects whatever.

7. Alternating currents of a three-inch Ruhmkorff coil

passed through bouillon cultures for ten hours favor growth and pigment production.

8. High frequency, high potential currents—Tesla currents—have neither antiseptic nor bactericidal properties when passed around a bacterial suspension within a solenoid. When exposed to the brush discharges, ozone is produced and kills the bacteria.

9. Bouillon and hydrocele-fluid cultures in test-tubes of non-resistant forms of bacteria could not be killed by Röntgen rays after forty-eight hours' exposure at a distance of 20 mm. from the tube.

10. Suspensions of bacteria in agar plates and exposed for four hours to the rays, according to Rieder's plan, were not killed.

11. Tubercular sputum exposed to the Röntgen rays for six hours, at a distance of 20 mm. from the tube, caused acute miliary tuberculosis of all the guinea-pigs inoculated with it.

12. Röntgen rays have no direct bactericidal properties. The clinical results must be explained by other factors, possibly the production of ozone, hypochlorous acid, extensive necrosis of the deeper layers of the skin, and phagocytosis. The action of the *x*-rays upon bacteria has been investigated by Bonome and Gros,* Pott,† and others. When the cultures are exposed to their action for prolonged periods, their vitality and virulence seem to be slightly diminished. They are not killed by the *x*-rays.

(c) **Movement.**—Rest seems to be the condition best adapted for micro-organismal development. Slow-flowing movements do not have much inhibitory action upon the growth of bacteria, but violent agitation, as by shaking a culture in a machine, may hinder or prevent it. This explains why rapidly flowing streams, whose currents are interrupted by falls and rapids, should, other things being equal, furnish a better drinking-water than a deep, still-flowing river.

Galli-Valerio‡ has shown, however, that agitation does not inhibit the growth of the anthrax, typhoid or colon bacilli or the pneumococcus, but sometimes facilitates it.

(d) **Association.**—**Symbiosis** is the vital association of

* "Giornal. med. del Regis Esercito," an 45, u. 6.
† "Lancet," vol. II, No. 21, 1897.
‡ "Centralbl. f. Bakt.," etc., I Orig. 4, XXXVII, Sept. 23, 1904, p. 151.

different species of bacteria by which mutual benefit to one or the other is brought about. **Antibiosis** is an association detrimental to one of the associated organisms. Bacterial growth is greatly modified by the association of different species. Coley found the streptococcus more active when combined with Bacillus prodigiosus; Pawlowski that mixed cultures of Bacillus anthracis and Bacillus prodigiosus were less virulent than pure cultures of anthrax; Meunier* that when the influenza bacillus of Pfeiffer is inoculated upon blood agar together with Staphylococcus aureus its growth is favored by a change which the staphylococci bring about in the hemoglobin.

A similar advantageous association has been pointed out by Sanarelli, who found that Bacillus icteroides grows best and retains its vitality longest when grown in company with certain of the molds.

Rarely, the presence of one species of micro-organism entirely eradicates another. Hankin found that Micrococcus ghadialli destroyed the typhoid and colon bacilli, and suggested the use of this coccus to purify waters polluted with typhoid.†

An interesting experimental study of the bacterial antagonisms with special reference to the Bacillus typhosus, that the student should read, is by W. D. Frost, and appears in the "Journal of Infectious Diseases," 1904, 1, p. 599.

(e) **Extremes of Temperature.**—According to Fränkel, bacteria will rarely grow below 16° and above 40° C., but Flügge has shown that Bacillus subtilis will grow very slowly at 6° C.; at 12.5° C. fission does not take place oftener than every four or five hours; at 25° C. fission occurs every three-quarters of an hour, and at 30° C. about every half hour.

A few forms of bacteria grow at very high temperatures (60°–70° C.) and are described as *thermophilic*. They are found in manure piles and in hot springs. Tsiklinsky ‡ has described two varieties of actinomyces and a mold that he cultivated from earth and found able to grow well at 48°–68° C., though not at all at the temperature of the room.

* Société de Biologie, Séance du 11 Juin, 1898; "La Semaine médicale," June 15, 1898.

† "Brit. Med. Jour.," Aug. 14, 1897, p. 418.

‡ "Russ. Archiv f. Path.," etc., Bd. v, June, 1898.

Most bacteria are killed by temperatures above 60°–75° C., but their spores can resist boiling water for some minutes, though killed by dry heat if exposed to 150° C. for an hour or to 175° C. for from five to ten minutes.

The resistance of low forms of life to low temperatures is most astonishing. Cold inhibits the growth of all bacteria, and immersion in freezing mixtures destroys many. Some adult bacteria and most spores seem capable of resisting almost any degree of cold. Ravenel * exposed anthrax spores to the action of liquid air for three hours, diphtheria bacilli for thirty minutes, typhoid bacilli for sixty minutes, and Bacillus prodigiosus for sixty minutes, the temperature of the cultures being reduced to about —312° F., yet in no case was the vegetative capability of all of the bacteria destroyed, and when transferred to fresh culture bouillon they grew normally. His researches corroborate those of Pictet and Yung and others.

To say that bacteria are not injured by cold is a mistake, as Sedgwick and Winslow † have found that when typhoid bacilli are frozen, the greater number of them are destroyed, and that subsequent development of the frozen cultures takes place from the few surviving organisms.

Bacteria usually grow best at the temperature of a comfortably heated room (17° C.), and are not affected by its occasional slight variations. Some, chiefly the pathogenic forms, are not cultivable except at the temperature of the body (37° C.); others, like the tubercle bacillus, grow best at a temperature a little above that of the normal body.

(f) **The Presence of Antiseptics, etc.**—The presence of chemic agents, especially certain of the mineral salts, in an otherwise perfectly suitable medium may completely inhibit the development of bacteria, and if added to grown cultures in greater concentration, destroy them. Such substances are spoken of as antiseptics in the former, germicides or disinfectants in the latter case. Bichlorid of mercury and carbolic acid are the most familiar examples of germicides.

Though these agents are supposed to operate in definite concentrations with almost unvarying result, Trambusti ‡

* "The Medical News," June 10, 1899.

† "Centralbl. f. Bakt. u. Parasitenk.," etc., May 26, 1900, Bd. XXVII, Nos. 18, 19, p. 684.

‡ "Lo Sperimentale," 1893–4.

found it possible to produce a tolerance to a certain amount of bichlorid of mercury by cultivating Friedländer's bacillus upon culture media containing gradually increasing amounts of the salt, until from 1–15,000 which inhibit ordinary cultures, it could accommodate itself to 1–2000.

A thorough knowledge of the biology of bacteria and the conditions prejudicial to their life is of great practical importance in enabling one to carry out intelligently clinical precautions such as the sterilization of instruments, surgical dressings, etc., and use sufficiently radical measures for the disinfection of the skin to be incised, as well as of the hands of the operator.

CONSEQUENCES OF MICRO-ORGANISMAL ENERGY.

According to their activities, bacteria are described as—

Zymogens, or bacteria of fermentation.
Saprogens, or bacteria of putrefaction.
Chromogens, or color producers.
Photogens, or phosphorescent bacteria.
Aerogens, or gas producers.
Pathogens, or disease producers.

The vital activities of bacteria occasion many well-known changes in nature. Thus, it is through their energies that by fermentative and putrefactive changes organic matter is gradually transformed from complex to simple compounds. It is by the energy of bacteria that foul waters are gradually purified, and while it is true that the presence of large numbers of bacteria in water detracts from its potability, the very bacteria that cause its condemnation ultimately effect its purification by exhausting the organic matter it contains in their own nutrition. In the modern treatment of sewage by the "septic tank" method, the organic matter contained in the water is consumed through the agency of anaerobic and aerobic bacteria, until its consumption leaves the water once more clear and pure, the no longer useful bacteria dying out as the nutrition becomes exhausted.

The promptness with which bacteria attack organic matter is seen in the changes brought about in foods, some of which are ruined in flavor or quality, though others are thought to be improved. Thus, the flavor of butter, sausage, and cheese, the aroma of wines, and many other important gustatory characteristics of our foods depend solely upon the activity of bacteria or other micro-organisms.

Many of these activities are harmless, and indeed advantageous, though the fact that they are not infrequently accompanied by chemic changes, some of which are poisonous, makes it necessary to watch and time their operations lest acridity, acidity, insipidity, or toxicity of the food replace the desired effect.

Briefly considered, the best known phenomena resulting from bacterial energy are as follows:

1. Fermentation.—Fermentation is a chemic transformation of carbohydrates resulting from the activity of micro-organisms. The alcoholic fermentation, which is a familiar phenomenon to the layman as well as to the brewer and chemist, depends upon the activity of a yeast-plant, one of the saccharomyces fungi. The acetic-acid, lactic-acid, and butyric-acid fermentations are caused by their respective bacilli (Bacillus aceticus, Bacillus acidi lactici, and Bacillus butyricus). A considerable number of bacilli seem capable of converting milk-sugar into lactic acid. There seems to be no specific micro-organism for the lactic-acid fermentation, although Bacillus acidi lactici is a powerful acid producer. There are also several bacteria which produce the acetic fermentation, though it is generally attributed to a special common form, Mycoderma aceti or Bacillus aceticus. The butyric fermentation generally due to Bacillus butyricus may also be caused by other bacilli. (For an exact description of the chemistry of the fermentations reference must be made to special textbooks.*)

2. Putrefaction.—Putrefaction is a chemic disintegration of nitrogenous compounds resulting from the activity of micro-organisms. The first step in the process seems to be the transformation of the albumins into peptones, then the splitting up of the peptones into gases, acids, bases, and salts. Both fermentative and putrefactive processes apparently take place through the agency of enzymes produced by the bacteria. In the process the innocuous albumins are frequently changed to toxalbumins, and sometimes to peculiar putrefactive alkaloids known as *ptomains*.

* See "Enzymes and Their Applications," by Jean Effront, translated by S. C. Prescott, New York, 1902; "Micro-organisms and Fermentation," by Alfred Jörgensen, translated by A. K. Miller and A. E. Lennholm, London, 1900; and the many writings of Christian Hansen.

TABLE OF THE PTOMAINS.—(*Vaughan and Novy.*)

Formula.	Name.	Discoverer.	Physiologic Action.
CH_5N.	Methylamin.	Bocklisch.	Non-poisonous.
C_2H_7N.	Dimethylamin.	Brieger.	"
C_3H_9N.	Trimethylamin.	Dessaignes.	"
C_2H_5N.	Spermin.	Kunz.	"
C_2H_7N.	Ethylamin.	Hesse.	"
$C_4H_{11}N$.	Diethylamin.	Bocklisch.	"
$C_6H_{15}N$.	Triethylamin.	Brieger.	"
C_3H_9N.	Propylamin.	"	. . .
$C_4H_{11}N$.	Butylamin.	Gautier and Mourgues.	Poisonous (?).
$C_5H_{11}N$.	Tetanotoxin.	Brieger.	"
$C_5H_{13}N$.	Amylamin.	Hesse.	"
$C_6H_{15}N$.	Hexylamin.	"	"
$C_7H_{11}N$.	Di-hydrolutidin.	Gautier and Mourgues.	"
$C_8H_{11}N$.	Collidin (?).	Nencki.	. . .
$C_8H_{11}N$.	Pyridin-base (?).	Gautier and Etard.	Poisonous (?).
$C_9H_{12}N$.	Parvolin (?).	" "	"
$C_{10}H_{16}N$.	Unnamed.	Guareschi and Mosso.	"
$C_{10}H_{15}N$.	Pyridin-base (?).	O. de Coninck.	. . .
$C_{32}H_{31}N$.	Unnamed.	Delezinier.	. . .
$C_2H_3N_3$.	Ethylidenediamin (?).	Brieger.	Poisonous (?).
$C_3H_6N_2$.	Anthrocin.	Hoffa (1889).	. . .
$C_3H_8N_2$.	Trimethylenediamin (?).	Brieger.	Poisonous (?).
$C_4H_{12}N_2$.	Putrescin.	"	Not very poisonous.
$C_5H_{14}N_2$.	Cadaverin.	"	Not very poisonous.
$C_5H_{14}N_2$.	Neuridin.	"	Non-poisonous.
$C_5H_{14}N_2$.	Saprin.	"	"
$C_6H_{16}N_2$.	Hexamethylenediamin.	Garcia.	. . .
$C_7H_{10}N_2$.	Unnamed.	Morin.	Non-poisonous.
$C_{10}H_{26}N_2$ (?).	Susatoxin.	Novy.	Poisonous.
$C_2H_7N_3$.	Methyl-guanidin.	Brieger.	"
$C_{19}H_{27}N_3$.	Morrhuin.	Gautier and Mourgues.	Diuretic, etc.
$C_{13}H_{20}N_4$.	Unnamed.	Aser.	. . .
$C_{17}H_{38}N_4$.	"	Gautier and Etard.	. . .
$C_{25}H_{32}N_4$.	Asellin.	Gautier and Mourgues.	Poisonous.
$C_5H_{13}NO$.	Neurin.	Brieger.	"
$C_8H_{11}NO$.	Mydin.	"	Non-poisonous.
$C_5H_{11}NO_2$.	δ-amido-valerianic acid.	E. and H. Salkowski.	"
$C_5H_{15}NO_2$.	Cholin.	Brieger.	Poisonous.
$C_6H_{13}NO_2$.	Mydatoxin.	"	"
$C_6H_{13}NO_2$.	Unnamed.	Brieger (1888, tetanus culture).	Non-poisonous.
$C_6H_{15}NO_2$.	Mytilotoxin.	Brieger.	Poisonous.
$C_7H_{17}NO_2$.	Godinin.	"	"
$C_7H_{17}NO_2$.	Typhotoxin.	"	"
$C_7H_{17}NO_2$.	Unnamed,	"	"
$C_{14}H_{14}N_2O$.	Pyocyamin.	Ledderhose.	Non-poisonous.
$C_5H_{13}NO_3$.	Betain.	Brieger.	"
$C_5H_{13}NO_3$.	Muscarin.	"	Poisonous.
$C_9H_{13}NO_3$.	Morrhuic acid.	Gautier and Mourgues.	. . .
$C_5H_{12}N_2O_4$.	Unnamed.	Pouchet.	Poisonous.
$C_{13}H_{30}N_2O_4$.	Tetanin.	Brieger.	"
$C_{14}H_{20}N_2O_4$.	Unnamed.	Guareschi.	. . .
$C_{16}H_{22}N_2O_4$.	"	Lepierre.	Poisonous.
$C_7H_{18}N_2O_6$.	"	Poucket.	"
. . .	Tyrotoxicon.	Vaughan.	"
. . .	Mydalein.	Brieger.	"
. . .	Spasmotoxin.	"	"
. . .	α-diamin (?).	Brieger (tetanus culture).	"
. . .	Peptotoxin.	Brieger.	"
. . .	Phlogosin.	Leber.	Inflammatory.

Ptomains.—Vaughan and Novy define a ptomain as "*a chemical compound, basic in character, formed by the action of bacteria on organic matter.*" The chemistry of these bodies is very complex, and for a satisfactory description of them Vaughan and Novy's book* is excellent.

Ptomains probably play but a small part in pathologic conditions. They are formed almost exclusively outside of the living body, and only become a source of danger when ingested with the food. It is supposed that cases of ice-cream and cheese-poisoning are usually due to *tyrotoxicon*, a ptomain produced by the putrefaction of the proteid substances of the milk before it is frozen into ice-cream or made into cheese. The safeguard is to freeze the milk only when perfectly fresh and avoid mixing the milk, cream, sugar, and flavoring substances, and allowing the mixture to stand for some time beforehand.

It is supposed that the occasional cases of "Fleischvergiftung," "meat-poisoning," or "Botulismus," are due to the development of toxic ptomains in consequence of the growth of certain bacteria (Bacillus botulinus) in the meat. Kaensche† carefully investigated the subject, and gives a synoptic table containing all the described bacteria of this class. His researches show that there are at least three different bacilli whose growth causes the development of poisonous ptomains in meat. In general these organisms resemble Bacillus coli.

With the increase of knowledge upon the toxic character of the bacteria themselves, the importance of the toxic ptomains has diminished, until at present we have come to regard them as very rare causes of disease.

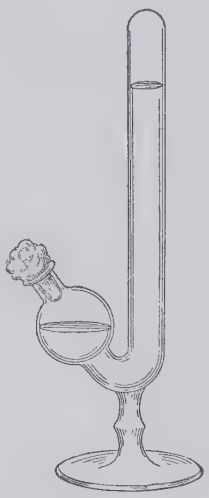


Fig. 5.—Smith's fermentation-tube.

3. Production of Gases.—Various gases are given off during decomposition and fermentation, among them being CO_2, H_2S, NH_4, H, CH_4, and others. Gases produced by aerobic bacteria usually fly off from the surface of the culture unno-

* "Ptomaïnes and Leucomaïnes," 1888; "Cellular Toxins," 1902.
† "Zeitschrift für Hygiene," etc., Bd. xxii, Heft 1, June 25, 1896.

ticed, but if the bacterium be anaerobic and develop at the lower part of a tube of culture media, a visible bubble of gas is usually formed about the colonies. Such gas bubbles are almost invariably present in cultures of the bacilli of tetanus and malignant edema.

To quantitatively determine the gas-production, some form of the Smith fermentation-tube is most convenient. The tube is filled with bouillon containing some sugar, sterilized as usual, inoculated, and stood aside to grow. As the gases form, the bubbles ascend and accumulate in the closed arm. In estimating quantitatively, one must be careful that the tube is not so constructed as to allow the gas to escape as well as to ascend into the main reservoir.

For the determination of the nature of the gases produced, Theobald Smith has recommended the following method:

"The bulb is completely filled with a 2 per cent. solution of sodium hydroxid (NaOH) and tightly closed with the thumb. The fluid is shaken thoroughly with the gas and allowed to flow back and forth from the bulb to the closed branch, and the reverse several times to insure intimate contact of the CO_2 with the alkali. Lastly, before removing the thumb all the gas is allowed to collect in the closed branch so that none may escape when the thumb is removed. If CO_2 be present, a partial vacuum in the closed branch causes the fluid to rise suddenly when the thumb is removed. After allowing the layer of foam to subside somewhat the space occupied by gas is again measured, and the difference between this amount and that measured before shaking with the sodium hydroxid solution gives the proportion of CO_2 absorbed. The explosive character of the residue is determined as follows: The cotton plug is replaced and the gas from the closed branch is allowed to flow into the bulb and mix with the air there present. The plug is then removed and a lighted match inserted into the mouth of the bulb. The intensity of the explosion varies with the amount of air present in the bulb. The relative proportion of gases resulting from the fermentation is frequently of importance for the differential diagnosis of related bacteria. Smith has designated this relation of $\frac{H}{CO_2}$ as the 'gas formula.' The colon bacillus has a gas formula corresponding to $\frac{H}{CO_2} = \frac{2}{1}$. Other aerogenic bacilli sometimes show a formula $\frac{H}{CO_2} = \frac{1}{2}$."

W. B. MEACHAM.

4. Liquefaction of Gelatin.—As certain bacteria grow in gelatin, the medium becomes partly or entirely liquefied. This peculiarity is apparently independent of any other property of the bacterium, and is manifested alike by pathogenic and non-pathogenic forms. The liquefaction is supposed to be dependent upon a form of peptonization. Bitter * and Sternberg † first showed that if from a culture in which liquefaction has taken place the bacteria be removed by filtration, the filtrate will retain the power of liquefying gelatin, showing the property is not resident in the bacteria, but in some substance in solution in their excreted products. These products were described as "tryptic enzymes" by Fermi,‡ who found that heat destroyed them. Mineral acids seem to check their power to act upon gelatin. Formalin renders the gelatin insoluble. Some of the bacteria liquefy the gelatin in such peculiar and characteristic manner as to make the appearance an extremely valuable guide for the differentiation of species.

5. Production of Acids and Alkalies.—Under the head of "Fermentation" the formation of acetic, lactic, and butyric acids has been discussed. Formic, propionic, baldrianic, palmitic, and margaric acids also result from microbic metabolism. As the acidity progresses, it impedes, and ultimately completely inhibits, the activity of the bacteria. The cultivation of the bacteria in milk to which litmus or lacmoid has been added is a convenient method for detecting changes of reaction. Rosolic acid solutions may also be used, the acid converting the red into an orange color. Neutral red is also much employed for this purpose, the acids of the colon bacillus turning it yellow.

The quantitative estimation of changes in reaction can be best made by titration, and the fermentation-tube culture can be employed for the purpose. The contents of the bulb and branch should be shaken together, a measured quantity withdrawn, and titration with $\frac{n}{20}$ sodium hydroxid, or $\frac{n}{20}$ hydrochloric acid, performed.

The alkali most frequently formed by bacterial growth is ammonium, which is set free from its combinations, and either flies off as a gas or forms new combinations

* "Archiv für Hygiene," 1886, Heft 2.
† "Medical News," 1887, No. 14.
‡ "Centralbl. f. Bakt.," etc., 1891, Bd. x, p. 401.

with acids simultaneously formed. Some bacteria produce acids only, some alkalies only, others both acids and alkalies. Both acids and the alkalies, when in excess, serve to check the further activity of the micro-organisms.

6. Chromogenesis.—Bacteria that produce colored colonies or impart color to the medium in which they grow are called *chromogenic;* those producing no color, *non-chromogenic.* Most chromogenic bacteria are saprophytic and non-pathogenic. Some of the pathogenic forms, as Staphylococcus pyogenes aureus, are, however, color producers. It seems more likely that certain chromogenetic substances unite with constituents of the culture medium to produce the colors than that the bacteria form the actual pigments; but, as Galeotti * has shown, there are two kinds of pigment, one being soluble, readily saturating the culture medium, as the pyocyanin and fluorescin of Bacillus pyocyaneus, the other insoluble, not tingeing the solid culture media, but retained in the colonies, like the pigment of Bacillus prodigiosus. The pigments are found in greatest intensity near the surface of a bacterial mass. The coloring matter never occupies the cytoplasm of the bacteria (except Bacillus prodigiosus, in whose cells occasional pigment-granules may be seen), but occurs as an intercellular excrementitious substance.

Almost all known colors are formed by different bacteria. One bacterium will sometimes elaborate two or more colors; thus, Bacillus pyocyaneus produces pyocyanin and fluorescin, both being soluble pigments—one blue, the other green. Gessard † has shown that when Bacillus pyocyaneus is cultivated upon white of egg, it produces only the green fluorescent pigment, but if cultivated in pure peptone solution it produces only the blue pyocyanin. His experiments prove the very interesting fact that for the production of fluorescin it is necessary that the culture medium contain a definite amount of a phosphatic salt. Sometimes, when an organism produces two pigments, one is soluble, the other insoluble, so that the colony will appear one color, the medium upon which it grows another. I once found an interesting coccus,‡ with this peculiarity, upon

* "Lo Sperimentale," 1892, XLVI, Fasc. III, p. 261.

† "Ann. de l'Inst. Pasteur," 1892, pp. 810–823.

‡ See Norris and Oliver, "System of Diseases of the Eye," vol. II, p. 489, and "University Medical Magazine," Sept. 1895.

the conjunctiva. It formed a brilliant yellow colony upon the surface of agar-agar, but colored the agar-agar itself a beautiful violet. In this case the yellow pigment was insoluble, the violet pigment very soluble and diffusible through the jelly. Some organisms will only produce pigments in the light; others, as Bacillus mycoides roseus, only in the dark. Some produce them only at the room temperature, but, though growing luxuriantly in the incubator, refuse to produce pigments at so high a temperature. Thus, Bacillus prodigiosus produces a brilliant red color when growing at the temperature of the room, but is colorless when grown in the incubator. The reaction of the culture medium is also of much importance in this connection. Thus, Bacillus prodigiosus produces an intense scarlet-red color upon alkaline and neutral media, but is colorless or pinkish upon slightly acid media. Colored lights seem to have no modifying influence upon the pigment-production. Even if for successive generations the bacterium be grown so as to be colorless, it speedily recovers its primitive color when restored to its old environment. I* have found that Bacillus prodigiosus, robbed of its color for many generations by incubation, when placed in the normal environment produces its original red pigment, no matter what color the light thrown upon it. Some of the pigments—perhaps most of them—are formed only in the presence of oxygen.

7. Production of Odors.—Gases such as H_2S and NH_2 have sufficiently characteristic odors. There are, however, a considerable number of pungent odors which seem dependent upon odoriferous principles independent of any gases. Many of them are extremely unpleasant, as that of the tetanus bacillus. The odors seem to be peculiar individual characteristics of the organisms.

8. Production of Phosphorescence.—Cultures of Bacillus phosphorescens and numerous other organisms are distinctly phosphorescent. So much light is sometimes given out by gelatin cultures of these bacteria as to enable one to see the face of a watch in a dark room. Gorham found the photogenesis most marked when the organisms are grown in alkaline media at *room* temperature. Most of the phosphorescent bacteria are found in sea-water, and are best cultivated in sea-water gelatin. Some are familiar to

* "University Medical Magazine," July, 1894, vol. VI, No. 10, p. 675.

butchers through the phosphorescence they cause on the surface of fresh meats.

9. Production of Aromatics.—Phenol, kresol, hydrochinone, hydroparacumaric acid, and paroxyphenylic-acetic acid are by no means uncommon products of bacteria. The most important is *indol,* which was at one time thought to be peculiar to the cholera spirillum, but is now known to be produced by many other bacteria. The best method of testing for it is that of Salkowski, known as the nitroso-indol reaction. To perform it, 10 c.c. of the fluid to be tested receive an addition of 1 c.c. of a 0.02 per cent. solution of potassium nitrite. The mixture is shaken in a test-tube. A few drops of concentrated sulphuric acid are then allowed to flow down the side of the tube and form a layer at the bottom. If indol is present, a purple-red color develops at the junction of the two fluids. McFarland and Small* have found that the intensity of this color corresponds to the quantity of indol present, and that quantitative tests can be made by means of a comparative color test series.

10. The Formation of Nitrates.—A process of fundamental importance is carried on by certain lowly bacteria of the soil. Since plants are unable to assimilate the free nitrogen of the air, but must obtain this element from the soil in the form of some soluble compound, and since there is a relatively limited amount of combined nitrogen in the world, it becomes of the last importance that the supplies which are continually withdrawn from the soil should be replaced by the nitrogen liberated in the decay of organic material. This nitrogen, after a series of putrefactive changes have occurred, appears as ammonia. The odor of this gas is often plainly perceptible about manure heaps. In this form nitrogen is poorly adapted for use by plants, and moreover may be easily dissipated. An extensive further process of oxidation is carried on by the nitrifying bacteria, whereby nitrates are ultimately formed. These are eminently adapted for use by plants, and so the soil is rendered continuously capable of supporting vegetation.

Nitrosomonas and nitrosococcus convert ammonia into nitrous acid, and nitrobacter oxidizes the latter to form nitric acid.

These genera are well nigh universal in the soil. They do

* "Trans. of the American Public Health Association," 1905.

not grow on the ordinary culture media, but require special solutions, free from the diffusible albumins,—free indeed from organic compounds of any sort! Their supplies of carbon are obtained by the dissociation of carbon dioxid. It is highly noteworthy that they are thus able to flourish without food more complex than ammonia, a fact which is without parallel among organisms devoid of chlorophyl.

11. Reduction of Nitrates.—A considerable number of bacteria are able to reduce nitrogen compounds in the soil or in culture media, prepared for them, into ammonia. To the horticulturist this matter is of much interest. Winogradsky* has described specific nitrifying bacilli which he found in soil, and asserts that the presence of ordinary bacteria in the soil causes no formation of nitrites so long as the special bacilli are withheld.

Reduction of nitrates can be determined experimentally by the use of a *nitrate broth* made by dissolving in 1000 c.c. of water, 1 gram of peptone and 0.2 gram of potassium nitrate. The ingredients are dissolved, filtered, then filled into tubes, and sterilized. The tubes are inoculated and the results noted. As nitrites and ammonia are, however, commonly present in the air and are taken up by fluids, it is always well to control the test by an uninoculated tube tested with the reagents in the same manner as the culture.

Two solutions are employed† for testing the culture:

I. Naphthylamin, 0.1 gram,
Distilled water, 20.0 grams,
} Boil, cool, filter, and add 156 c.c. of dilute (1 : 16) hydric acetate.

II. Sulphanilic acid, 0.5 gram.
Hydric acetate, diluted, 150.0 c.c.

Keep the solutions in glass-stoppered bottles and mix equal parts for use at the time of employment.

About 3 c.c. of the culture and an equal quantity of the uninoculated culture fluid are placed in test-tubes and about 2 c.c. of the test fluid slowly added to each. The development of a red color indicates the presence of nitrites, the intensity of the color being in proportion to the quantity of nitrites present. If a very slight pinkish or reddish color in the uninoculated culture fluid and a deeper red in the culture develop, it shows that a small amount of

* "Ann. de l'Inst. Pasteur," 1891; "La Semaine médicale," 1892.

† "Journal of the American Public Health Association," 1888, p. 92.

nitrites was already present, but that more have been produced by the growth of the bacteria.

The presence of ammonia in either fluid is easily determined by the immediate development of a yellow color or precipitate when a few drops of Nessler's solution* are added.

Failure to determine either ammonia or nitrites may not mean that the nitrates were not reduced, but that they were reduced to N. It is, therefore, necessary to test the solutions for nitrates, which is done by the use of phenol-sulphonic acid and sodium hydroxid, which in the presence of nitrates give a yellow color.

12. Combination of Nitrogen.—Not only do bacteria destroy or reduce nitrogen compounds, but some of them are also able to assimilate nitrogen from the air and combine it so as to be useful for the nourishment of vegetable and animal life. The most interesting organisms of this kind are found upon the roots of the leguminous plants, peas, clover, etc., and have been studied by Beyerinck.† It seems to be by the entrance of these bacteria into their roots that the plants are able to assimilate nitrogen from the atmosphere and enrich sterile ground. Every agriculturist knows how sterile soil is improved by turning under one or two crops of clover with the plough.

13. Peptonization of Milk.—Numerous bacteria possess the power of digesting—peptonizing—the casein of milk. The process varies with different bacteria, some digesting the casein without any apparent change in the milk, some producing coagulation, some gelatinization of the fluid. In some cases the digestion of the casein is so complete as to transform the milk into a transparent watery fluid.

Milk invariably contains large numbers of bacteria, that enter it from the dust of the dairy, many of them possessing this power and ultimately spoiling the milk. In the process of peptonization the milk may become bitter, but need not change its original reaction.

The phenomena of coagulation and digestion of milk can be made practical use of to aid in the separation of similar species of bacteria. Thus, the colon bacillus coagulates milk, but the typhoid bacillus does not.

* Nessler's solution consists of potassium iodid, 5 grams, dissolved in hot water, 5 c.c. Add mercuric chlorid, 2.5 grams, dissolved in 10 c.c. of water, then to the mixture add potassium hydrate, 16 grams, dissolved in water, 40 c.c., and dilute the whole to 1000 c.c.

† See "Centralbl. f. Bakt.," etc., Bd. VII, p. 338.

14. Production of Disease.—Bacteria that produce disease are known as *pathogenic;* those that do not, as *non-pathogenic.* Between the two groups there is no sharp line of separation, for true pathogens may be cultivated under such adverse conditions that their virulence may be entirely lost, while bacteria ordinarily harmless may be made virulent by certain manipulations. In order to determine that a micro-organism is possessed of pathogenic powers, the committee of bacteriologists of the American Public Health Association* recommends that: (1) When a given form grows only at or below 18°–20° C., inoculation of about 1 per cent. of the body-weight with a liquid culture seven days old should be made into the dorsal lymph-sac of a frog. (2) When a species grows at 25° C. and upward, an inoculation should be made into the peritoneal cavity of the most susceptible (in general) of warm-blooded animals —*i. e.*, the mouse, either the white or the ordinary house mouse. The inoculation should consist of about 1 per cent. of the body-weight of the mouse of a four- to eight-hour standard bouillon culture, or a broth or water suspension of one platinum loop from solid cultures. When such intraperitoneal injection fails, it is unlikely that other methods of inoculation will be successful in causing the death of the mouse. If the inoculations of the frog and mouse both prove negative, the committee think it unnecessary to insist upon any further tests of pathogenesis as being requisite for work in species differentiation.

Production of Enzymes by Bacteria.—Some of these have already been mentioned as causing fermentation and putrefaction, coagulating milk, dissolving gelatin, etc. There are, however, others which have interesting and important actions upon both animal and vegetable substances.

Knowledge upon the subject is just becoming systematized, one of the best writings being by Emmerich and Löw,† who observed that in old cultures of Bacillus pyocyaneus the bacteria become transformed into a gelatinous mass, and were led to experiment with old and degenerating cultures condensed to $\frac{1}{10}$ volume in a vacuum apparatus. The bacteriolytic powers were then found to be much increased, and they were subsequently able to precipitate from the concentrated culture an enzyme, which they

* "Jour. Amer. Public Health Assoc.," Jan., 1898.

† "Zeitschrift für Hygiene," 1899.

called *pyocyanase*. The authors reach the rather hasty conclusions that the cessation of growth of bacteria in cultures depends upon the generation of enzymes; that the enzymes destroy the dead bacteria; that the enzymes will kill and dissolve living bacteria and destroy toxins, and, therefore, are useful for the treatment of infectious diseases; and that antitoxins are simply accumulated enzymes which the immunized animals have received during treatment, and which, appearing in the serum, produce the effects so well known.

It is probable that many of the toxic effects of bacteria and their cultures depend upon enzymic substances, the nature of which we do not yet understand.

CHAPTER III.

INFECTION.

INFECTION is the successful invasion of the organism by parasites. Unfortunately custom has partly sanctioned the use of the word infection in various other and sometimes confusing senses. Thus, a table or knife upon which bacteria are known to be or are even supposed to be present, is said by the surgeon to be "infected," when infective might better convey the meaning intended. The mouth and intestine, which naturally harbor numerous bacteria of various forms, are also spoken of by the surgeon as "infected," when no abnormal condition is present. A splinter penetrating the skin, and carrying harmless bacteria into the deeper tissues, is said to infect them, when, in fact, none of the phenomena of infection result.

Infection should always imply an abnormal state resulting from the deleterious action of the parasite upon the host. The colon bacillus is a constant inhabitant of the intestine of every human being, and of most of the lower animals, but under ordinary conditions does no harm. The intestine is, therefore, not "infected" but infested with it, and it is only when abnormal or unnatural conditions arise that infection can take place. The constant association of certain bacteria with certain parts of the body to which they ordinarily do no harm, but into which they may rapidly invade when appropriate conditions arise, may best be described, as suggested by Adami, as *sub-infection*. The potentiality is always there, though active invasion is but rarely if ever accomplished.

There are two inseparable factors to be considered in all infections: the organism *infecting*, and the organism *infected*. The first is the *parasite*, the second, the *host*. Infectivity and infectability may depend upon peculiarities of either parasite or host. Organisms that have lived together in a neutral relationship for an almost indefinite period may suddenly cease their customary association, because of

newly acquired power of invasion on the one hand, or diminished vital resistance on the other, and infection take place where it had previously been impossible.

Bacteria are commonly called *saprophytic* when they live in nature apart from other living organisms, and *parasitic* when they live in or upon them. Saprophytic bacteria when accidentally transplanted from their natural environment to the body of some animal, for example, may or may not be capable of continuing life under the new conditions. In the greater number of cases they die, but sometimes the new environment seems better than the old, and they multiply rapidly, invade the tissues in all directions, eliminate their metabolic products into the juices, and occasion varying morbid conditions.

The parasitic bacteria live in habitual association with higher organisms. Sometimes, and indeed most commonly, it is a harmless association, like that of certain cocci upon the skin, of the colon bacillus in the intestine, etc., but occasionally it results in the destruction of the tissues and the final dissolution of the host, as in tuberculosis, leprosy, etc.

Pathogenic bacteria are associated with the production of morbid conditions. They may be saprophytes accidentally admitted to the body, or they may be parasites unknown except in the body of the host. The group of pathogenic organisms has no well-defined limits, for it is frequently observed that micro-organisms well known under other conditions, and not known to have been engaged in pathogenic processes, turn up unexpectedly as the cause of some morbid condition. Indeed, although we are acquainted with a large number of organisms that have never been observed in connection with disease, we are scarcely justified in concluding that they are incapable of producing injury should proper conditions arise.

SOURCES OF INFECTION.

The sources of infection may be *exogenous* or *endogenous;* that is, they may arise through the admission to the tissues of micro-organisms from sources entirely apart from the individual infected, or through the admission of some of those parasitic and usually harmless organisms constantly associated with him.

Exogenous infections arise from accidental contact with the external world.

A polluted *atmosphere* may carry into the respiratory passages micro-organisms capable of colonizing there. Polluted *water* or *food* may carry into the intestine micro-organisms whose temporary residence may entirely change the functional and structural integrity of the parts.

Wounds inflicted by the teeth of animals, by weapons, by implements, or by objects of various kinds, carry into the tissues micro-organisms whose operations, local or general, may variously affect the organism to its detriment.

The use of *unclean objects* of various kinds—spoons, knives, cups, blow-pipes, catheters, syringes, dental instruments, etc.—may serve to transfer disease-producing organisms from one person to another who might otherwise never come in contact with them.

Suctorial insects seem occasionally to act as the medium by which micro-organisms being withdrawn in blood from one person may be introduced into other persons so that they become infected. The flea is supposed by many to thus aid in the spread of plague.

Fomites, or objects made infective through contact with individuals suffering from smallpox, scarlatina, and other contagious or actively infectious diseases, become the means through which the specific micro-organisms may be conveyed to the well with resulting infection.

Endogenous infections arise through the activity of micro-organisms habitual to the body itself. It always indicates a morbid condition of the body by which the defensive mechanisms are disturbed so that organisms harmless under normal conditions become invasive.

All normal animals are born free of parasitic micro-organisms, but it is impossible for them to remain so because of the universal distribution of micro-organismal life. The air, the water, the soil, and the food, as well as the associates of the young animal, all act as means by which micro-organisms, and especially bacteria, are brought to the surface and cavities of its body, and but a short time elapses after its birth before it harbors the customary parasitic forms.

BACTERIAL TENANTS OF THE NORMAL HUMAN BODY.

The skin and adjacent mucous membranes. The slightly moist warm surface of the skin is well adapted to bacterial life, and its unavoidable contact with surrounding objects determines that a great variety of organisms shall

adhere to it. Of these, we can differentiate between forms whose presence is unexpected and temporary; others whose presence may be expected; and still others whose presence is invariable.

Elaborate investigations upon the bacterial flora of the skin have been made by Unna*; Mittman,† who studied the finger-nails, under which he found no less than seventy-eight different species; Maggiora,‡ who isolated twenty-nine forms from the skin of the foot; and Preindelsberger,§ who found eighty species of bacteria on the hands. Undoubtedly many of these organisms were accidentally present, and were at least only semi-parasitic. Not a few were met but once and were in no sense bacteria of the skin. The skin may also be temporarily contaminated with bacteria from other portions of the patient's body, as, for instance, from his intestine; thus Winslow‖ has found the colon bacillus upon the hands of ten out of one hundred and eleven persons examined. Wigura** also examined the hands of forty persons in hospitals, finding tubercle bacilli in two out of ten persons from phthisical wards in hospitals, colon bacilli six times and typhoid bacilli once on the hands of nine attendants in the typhoid wards. He found streptococci and staphylococci many times. Welch†† and Robb and Ghriskey‡‡ seem to have been the first to make a clear differentiation between the accidentally present bacteria and the permanently parasitic organisms of the skin, and to show that certain cocci, producing white and yellow colonies upon agar-agar, were invariable in occurrence and penetrated to the lowest epidermal layers.

These cocci, of which Welch describes the most common

*"Monatshefte für prakt. Dermatol.," VII, 1888, p. 817; VIII, 1889, pp. 293, 562; IX, 1889, p. 49; X, 1890, p. 485; XI, 1890, p. 471; XII, 1891, p. 249.

†"Archiv. f. path. Anat. u. Phys. u. f. klin. Med.," CXIII, 1888, p. 203.

‡"Giornale della R. Societá d'Igiena," 1889, Fasc. 5, p. 335.

§"Samml. Medic. Schriften," herausg. von der "Wiener klin. Wochenschrift," XXII, Wien, 1891; "Rev. Jahresbericht über die Fortschritten in der Lehre von den pathogenen Mikroorganismen," VII, 1891, p. 619.

‖"Jour. Med. Research," vol. X, p. 463.

**"Wratsch," 1895, No. 14.

††"Transactions of the Congress of American Physicians and Surgeons," II, 1891, p. 1.

‡‡"Bulletin of the Johns Hopkins Hospital," III, 1892, p. 37.

as Staphylococcus epidermidis albus, are universally and invariably present upon the human skin, and must be regarded as habitual parasites.

Where the skin is peculiar in its moisture and greasiness, however, additional forms are found. Thus, in preputial smegma, in the axillæ, and sometimes about the lips and nostrils, a bacillary organism, Bacillus smegmatis, is invariable, and the recent work by Schaudinn and Hoffman* has shown that the skin of the genitalia harbors a spiral organism which they call Spirochæta refringens.

In the *external auditory meatus* a coccus, Micrococcus cereus flavus, is almost always to be found in the waxy secretion.

Upon the *conjunctiva* as many accidental organisms may be found as shall have been caught by its moist surface, though the researches of Hildebrand and Bernheim and others seem to show that the tears have some antiseptic power and prevent the organisms from growing, so that in health there are very few permanent residents of the sac, certain cocci seeming to be the only constant forms.

The **mouth** has been carefully studied bacteriologically by Miller,† who found six organisms—Leptothrix innominata, Bacillus buccalis maximus, Leptothrix buccalis maxima, Iodococcus vaginatus, Spirillum sputigenum and Spirochæta dentinum (denticola)—in every mouth. These organisms are peculiar in that they will not grow in artificial culture. In addition to this permanent flora, Miller cultivated fifty-two other species, some of which were harmless, some well-known pathogens. From the mouth these organisms may be traced into the pharynx and esophagus.

The **stomach** seems to retain very few of the many bacteria that must enter it, its persistently acid contents being inimical to their development. Certain sarcina, especially Sarcina ventriculi, may be found without any considerable departure from the normal state. In carcinoma and other forms of pyloric obstruction with dilatation, the bacterial flora increases, and in achlorhydria micro-organisms of fermentation—Bacillus acidi lactici and Bacillus butyricus—make their appearance. They are, however, accidental and not permanent tenants of the organ.

The **intestine** receives such micro-organisms as have

* "Deutsche med. Woch.," May 4, 1905.

† "Micro-organisms of the Human Mouth," Phila., 1890.

survived whatever destructive influences the gastric juices may have exerted, and its alkaline contents, rich in proteids and carbohydrates in solution, are eminently appropriate for bacterial life. The flora of the intestine is, therefore, very large both in the number and variety of organisms present. The total number of permanent residents is not known. During infancy the predominating organism seems to be Bacillus lactis aerogenes; during adult life, Bacillus coli. Streptococci, especially Streptococcus coli gracilis, are also very common, if not invariable, inhabitants of the intestine.

The **vagina,** on account of its acid secretions, harbors

Fig. 6.—Sarcina ventriculi (Migula).

but few permanent forms of bacterial life. Bacillus acidi lactici is the micro-organism most commonly present.

The **uterus** harbors no bacteria in health, and but few in disease. The intervening acidity of the vagina makes it difficult for bacteria from the surface to penetrate so deeply, and the tenacious alkaline mucus of the cervix is an additional barrier to their progress. Careful studies of the bacteriology of the uterine secretions have been made by Gottschalk and Immerwahr.*

The **urethra** harbors a few cocci which enter the meatus from the surface and remain local in distribution.

The normal **bladder** is free from bacteria.

* "Archiv f. Gynäk.," 1896, Bd. L, Heft 3.

The **nose** constantly receives enormous numbers of bacteria in the dust of the inspired atmosphere. These organisms are too numerous and too various to mention, and might, indeed, comprehend the entire bacterial flora. But in spite of the large numbers of organisms received, the nose retains scarcely any, its mucous membranes seeming to be provided with some means of disposing of the organisms. Thompson and Hewlett* performed an interesting experiment to show this destructive action. Selecting the surface of one of the turbinated bones, they spread a culture of Bacillus prodigiosus upon it, and then transplanted from the surface at frequent intervals during a period of two hours. Cultures made within five minutes showed confluent colonies of the bacteria, but as time went on, the colonies became less and less numerous, until after two hours no more were found, all of the bacteria applied to the nose having been destroyed. These experiments confirm the opinion of Wurtz and Lermoyez that the nasal secretions possess germicidal powers. For these reasons the nose has no permanent parasitic flora.

The **larynx** and **trachea** contain very few bacteria and probably have no permanent parasitic flora.

The **lungs** harbor no bacteria. A few micro-organisms doubtless reach them in the inspired air, but the defensive mechanisms soon dispose of them, and the normal lung is almost invariably free of micro-organismal life.

AVENUES OF INFECTION.

The **skin** seems to form an effectual barrier against the entrance of bacteria into the deeper tissues. A few higher fungi—Trycophyton, Microsporon, Achorion, etc.—seem able to establish themselves in the superficial layers of the cells, invade the hair-follicles, and so reach the deeper layers, where morbid changes are produced. The minute size of the bacteria makes it possible for them to enter through lesions too small to be noticed. Garré applied a pure culture of Staphylococcus pyogenes aureus to the skin of his forearm, and found that furuncles developed in four days, though the skin was supposed to be uninjured. Bockhart moistened his skin with a suspension of the same organism, gently scratched it with his finger-nail, and suffered from a furuncle some days later.

The greater number of surgical infections result from the

* "Lancet," 1896.

entrance of bacteria through lesions of the skin. It makes but little difference to what depth the lesion extends,—abrasions, punctures, lacerations, incisions,—the protective covering is gone and the infecting organisms find themselves in the tissues, surrounded by the tissue lymph, under conditions appropriate for growth and multiplication, provided no inhibiting or destructive mechanism be called into action.

The **digestive apparatus** is the portal through which many infections take place. The Bacillus diphtheriæ, finding its way to the pharynx, speedily establishes itself upon the surface, producing pseudomembranous inflammation there. Typhoid bacilli, dysentery bacilli, cholera spirilla and related organisms, finding their way to the intestine, where the vital conditions are appropriate, take up their temporary residence there, to the inconvenience of the host, who suffers from one of the respective infections.

Various organisms pass from the pharynx to the tonsils and so to the lymph nodes and deeper tissues of the neck, where their first operations may be observed.

It is supposed by some pathologists that the digestive tract is a constant menace to health in that it regularly admits bacteria, through the lacteals, and perhaps through its capillaries, to the blood, where under slightly abnormal conditions they might do harm. According to Adami,* the intestine is responsible for a condition of sub-infection depending upon the constant entrance of colon bacilli into the blood. He finds the colon bacillus in the blood, and traces it to the liver, where its final dissolution takes place in the fine dumbbell-like granules enclosed in the cells. Nicholls† confirms Adami by finding similar dumbbell or diplococcoid bodies in the epithelial denuded tissues of the mesentery of normal animals.

Nicholas and Descos‡ and Ravenel§ fed fasting dogs upon a soup containing quantities of tubercle bacilli, killed them three hours later, and examined the contents of the thoracic duct, where tubercle bacilli, some alive and some dead, were found in large numbers. van Steenberghe and Grysez‖

* "Jour. of the American Medical Association," Dec. 16 and 23, 1899, vol. XXXIII, Nov. 25 and 26.

† "Jour. Med. Research," vol. XI, No. 2.

‡ "Jour. de Phys. et Path. gén.," 1902, IV, 910–912.

§ "Jour. Med. Research," X, p. 460, 1904.

‖ "Ann. de l'Inst. Pasteur," Tome XIX, No. 12, p. 787, Dec. 25, 1905.

found that carbon particles readily passed through the intestinal mucosa, entered the lymphatics and were so thrown into the venous circulation and so carried to the lung, this being the common method by which anthracosis was produced.

There are enough of these experiments to make it probable that the wall of the intestine is permeable to bacteria, and that in small numbers they constantly enter the blood of healthy animals, to be disposed of by mechanisms yet to be described.

Many of the bacteria penetrating the intestine must be retained in the lymph nodes; others, as in the experiment with the tubercle bacilli, meet destruction before they reach the blood; the remainder must reach the blood alive.

The presence of colon bacilli in the greater number of the organs shortly after death has led some pathologists to assume that they readily pass through the intestinal walls during the death agony, but although experiments have been made to prove and to disprove it, the matter is still controversial. Undoubtedly in the final dissolution some change takes place in the constitution of the individual by which general invasion by bacteria is made more easy than under normal conditions.

The **respiratory apparatus** affords admission to a few micro-organisms whose activities seem more easily carried on there than elsewhere. Although it is still controversial whether the inhalation of tubercle bacilli is as frequent a mode of conveying that organism into the body as was once supposed, it cannot be denied that its inhalation will account for the far greater frequency with which tuberculosis affects the lungs than other organs of the body.

Pneumonia, caused in an immense majority of cases by the pneumococcus of Fraenkel and Weichselbaum, probably results from the entrance of the organism into the respiratory tissues directly.

The entrance of the unknown infectious agents causing measles, German measles, smallpox, and scarlatina, can best be accounted for by supposing that they are inhaled into the lungs and thus enter the blood.

The **genital apparatus** is the portal of entry of micro-organisms whose early or chief operations are local. Among these are the gonococcus, which causes urethritis, vaginitis, balanitis, posthitis, endometritis, orchitis, salpingitis, vesicu-

litis, cystitis, oöphoritis, sometimes peritonitis and rarely endocarditis; the bacillus of Ducrey, that causes the chancroid or soft sore; and the micro-organism of syphilis, whatever it may be. In more rare cases other organisms, such as the common cocci of suppuration and the tubercle bacillus, may also be transmitted from individual to individual by sexual contact.

The **placenta** usually forms a barrier through which infectious agents find their way with difficulty. Some micro-organisms, however, readily pass through, and a few diseases such as syphilis are well known in the hereditary, congenital form. Pregnant women suffering from smallpox may be delivered of infants with marks indicative of prenatal disease. Some common infectious agents, such as the tubercle bacillus, seem to infect unborn animals with difficulty. The frequency of antenatal tuberculous infection is, however, somewhat controversial at present, Baumgarten having reached the opinion, exactly the opposite of what is commonly believed, that most children are subject to antenatal infection, though the bacilli subsequently develop and cause disease in only a few of them.

MODES OF BACTERIAL PATHOGENESIS.

This subject can be understood only through a broad knowledge of the metabolic products of the bacteria. In general it may be said that the ability of micro-organisms to do harm depends upon the injurious nature of their products. This alone, however, will not explain the phenomena of infection, for in many cases the intoxication is subsidiary in importance to the invasive power of the micro-organisms. Some bacteria having but limited toxic powers, possess extraordinary powers of invasion, as B. anthracis, and the intoxication becomes important only after the organisms have penetrated to all the tissues of the body. Others, with more active toxic properties, have but limited invasive powers, and a few organisms, growing with difficulty in some insignificant focus, excite actively destructive reactions in the tissues with which they come into contact. Still others, with limited invasive powers, eliminate active toxic substances, soluble in nature, that enter the circulation and act upon cells remote from the bacteria themselves, as in diphtheria and tetanus.

The *invasive power* of the bacteria depends upon their

ability to overcome the body defenses. This may indicate activity of the infecting organism, or weakness of the defensive mechanism. The relation of these factors is exceedingly complex, only partly understood, and will be fully discussed in the chapter upon Immunity.

The *toxic power of the bacteria* is intrinsic in the micro-organisms themselves.

For convenience toxins may be described as *intracellular* or insoluble, and *extracellular* or soluble.

The **intracellular toxins.** These products are but little known and have only recently begun to attract attention. Their insoluble nature makes it difficult to isolate them, and determines the limitations of their activity. Until the investigations of Vaughan, Cooley and Gelston,* and later Vaughan and his associates, Detweiler,† Wheeler,‡ Leach,§ Marshall and Gelston,‖ Gelston,** and J. V. Vaughan,†† Wheeler,‡‡ Leach,§§ McIntyre,‖‖ and others, it seemed remarkable that micro-organisms whose filtered cultures contained little demonstrable toxic substance are sometimes able to produce active pathologic activities. By means of special apparatus in which the micro-organisms could be cultivated in enormous quantities, and the disintegration of the micro-organismal masses secured by subjecting them to high temperatures or to the action of mineral acids, it was discovered that the colon bacilli, typhoid bacilli, and many supposedly harmless bacteria, contain intensely active toxic substances. In all probability some of the toxic substances produced by such means are artefacts, but enough work has been done to prove that insoluble toxic substances are undoubtedly present in such organisms, and the toxic substances obtained by the comminution of culture masses made solid and brittle by exposure to liquid air, as suggested by Macfadyen and Rowland; the autolytic digestion of bacteria washed free of their culture fluids and suspended in physiological salt solution, and the dissolution of bacteria by bacteriolytic animal juices have corroborated the original conclusions.

* "Journal of the American Medical Association," Feb. 23, 1901; "Trans. Assoc. Amer. Phys.," 1901, and "American Medicine," May, 1901.

† "Trans. Assoc. Amer. Phys.," 1902. ‡ Ibid.

§ Ibid. ‖ Ibid. ** Ibid.

†† Ibid. ‡‡ "Jour. Amer. Med. Assoc.," 1904, XLII, p. 1000.

§§ Ibid., p. 1003. ‖‖ Ibid., p. 1073.

It seems probable that there is considerable difference in the readiness with which these intracellular toxic substances are given up by the bacteria. From some they seem never to be set free in the bodies of animals into which the bacteria are injected; thus, Bacillus prodigiosus is harmless for animals, no matter what quantity is injected, yet active toxic substances can be extracted from the bodies of these organisms by appropriate chemical means. From others they are given off in small quantities either during the life of the organism, or at the moment of death and dissolution, as in the case of the typhoid bacillus and streptococci, whose filtered cultures are almost harmless, though both organisms are pathogenic.

The intracellular toxins are limited in action by the distribution of the bacteria producing them. When these organisms are but slightly invasive, more or less local reaction is produced; when they are actively invasive, general reactions of varying intensity result.

The **extracellular toxins,** of which those of Bacillus tetani and Bacillus diphtheriæ can be taken as types, have been known since the early work of Brieger and Fraenkel and Roux and Yersin. They seem to be excretions of the bacteria, which are not retained within the cells, but eliminated from them as rapidly as they are formed. Thus, in appropriate bouillon cultures of the diphtheria bacillus, the diphtheria toxin is present in large quantity and is highly virulent, but if the fluid be removed from the bacteria by porcelain filtration and the remaining bacilli carefully washed, their bodies are found to be devoid of similar toxic powers. The poison is most active where its diffusion is most limited. Agar-agar cultures of the tetanus bacillus are much more toxic than bouillon cultures because the soluble principle readily diffuses through the fluid but is held by the less diffusible agar-agar.

The soluble toxin is but one of numerous metabolic products of the bacteria. Thus in culture filtrates of the tetanus bacillus there are at least two very different active substances, the *tetano-spasmin* that acts upon the nervous system with convulsive effect, and the *tetano-lysin* that is solvent for erythrocytes.

In all probability all of the culture filtrates of bacteria are highly complex because of the addition of the various metabolic products—toxins, lysins, enzymes, pigments, acids,

etc.—of the bacteria, as well as because of changes produced in the medium by the abstraction of those molecular constituents upon which the bacteria have fed. This complexity makes it difficult to accurately study the toxins, which we scarcely know apart from their associated products.

The chemic nature of the toxins differs. Undoubtedly some are tox-albumins, but others are of different composition and fail to give the reactions belonging to the compounds of this group.

The variations observed in toxicogenesis under experimental conditions in the test-tube indicate that similar variations occur in the bodies of animals, and a few experiments conducted with slight variations in the composition and reaction of the media in which the bacteria grow will suffice to show that the exact effect of toxicogenic bacteria in the bodies of different animals cannot always be accurately prejudged.

The physiologic and pathogenic action of the extracellular soluble toxins differs from that of the intracellular and difficultly soluble toxins in that it is more easily diffused throughout the animal juices, and that its diffusion is independent of the invasiveness of the bacteria, so that a few organisms growing at some focus of unimportant magnitude, and causing but little local manifestation, may be able to produce a profound impression upon remote organs. This is best exemplified in the case of the Bacillus tetani, which, finding its way into the tissues under proper conditions, produces scarcely any local reaction,—indeed, the lesion may be undiscoverable,—yet may cause the death of the animal through the intensity of its action upon the central nervous system.

SPECIFIC ACTION OF BACTERIO-TOXINS.

The metabolic products of the greater number of injurious bacteria are characterized by irritative action upon those body cells with which they come into contact. If through the intracellular nature of the poisons and the mildly invasive character of the micro-organisms this action is localized at the seat of original infection, a local manifestation will result. Its exact nature will, however, be modified to some extent by other qualities of the bacterial products. Thus, when in addition to their irritative action, the products are strongly chemotactic, suppuration will follow.

The primary irritation is therefore a quality which many bacteria share in common, but the secondary suppuration, one peculiar to a few.

Irritation and suppuration are therefore non-specific actions, because numerous micro-organisms share the qualities productive of these conditions in common.

If the bacteria are rapidly invasive, but still have injurious products of the intracellular variety, they are apt to share certain qualities, such as the swelling of the lymph nodes, etc., in common, so that such lesions cannot be considered as specific. So soon as any one of the products is discovered to give some single lesion peculiar to that organism by which it is produced, or so soon as the total results of the activity of the various products of any micro-organism produce a typical result, differing from the total results of the operation of other micro-organisms, so that a recognized type of disease results, it becomes possible to say that the micro-organism in question is specific.

The most striking examples of the specific action of bacterio-toxins is, however, seen in those cases where soluble extracellular metabolic products of bacterial energy are liberated into the body juices so as to be conveyed by the circulatory system to all parts of the body. Those cells most susceptible to its action are then first or most profoundly impressed by it, and distinct physiological activities brought about. Thus, the soluble toxin of tetanus causes no visible reaction in the cells with which it first comes into contact at the seat of primary infection, because these cells are either less susceptible to its influence, or are less well able to show its effects, than remote cells of the nervous system to which it is secondarily carried by the blood. The cells of the connective tissue in which the tetanus bacillus is living show little reaction, but the motor cells of the central nervous system are profoundly impressed, and convulsions of the controlled muscular system are brought about. This special excitation of the nerve cells is specific because no other bacterio-toxin is known to produce it and it is attributed to special selective affinities of the nerve cells for the poison. This affinity has its analogue among the poisons of higher plants, thus, strychnin has a similar selective affinity and is also said to be specific in action upon the motor cells.

The venoms of various serpents, especially the cobra, also

have specific reactions, the cells of the respiratory centers seeming to be most profoundly affected by them.

The diphtheria bacillus, when observed in ordinary throat infections, is seen to produce a pseudomembranous angina. This results in part from the irritative local action of the organism, which it shares in common with many others, and in part from some coagulating product which it shares in common with a few—pneumococcus, streptococcus, etc. Neither of these reactions is specific, but subsequent to these early manifestations comes depressant action on the nervous cells with palsy, peculiar to the products of the diphtheria bacillus, and therefore specific.

It is upon the peculiar specific reactions of the bacteriotoxins that their ability to produce distinctive clinical conditions depends, and that we are able to recognize them as the essential factors in the production of any disease.

THE INVASION OF THE BODY BY BACTERIA.

Some bacteria whose invasiveness is insufficient to enable them successfully to maintain life in healthy tissues, occasionally get a foothold in diseased tissues and assist in morbid changes. This is sometimes seen in what is described by the surgeons as *sapremia*, in which various saprophytic bacteria, possessing no invasive powers, by growing in the putrefying tissues of a gangrenous part, give rise to poisonous substances which when absorbed by the adjacent healthy tissues produce constitutional disturbances, such as depression, fever, and the like.

Bacteria with limited invasive powers and intracellular toxins can at best occasion local inflammatory effects. Such organisms not infrequently vary, however, and when of unusual activity may survive entrance into the blood and lymph circulations and occasion *bacteremia*, or, as it is more frequently called, *septicemia*, a morbid condition characterized by the presence of bacteria in the circulating blood.

The mode by which this entrance of bacteria into the circulation is effected, differs in different cases. Kruse* believes that they sometimes are passively forced through the stomata of the vessels when the pressure of the inflammatory exudate is greater than that of the blood within them; that they may sometimes enter in the bodies of leukocytes that have incorporated them; that they may

* Flügge, "Die Mikroorganismen," vol. 1, p. 271.

actually grow through the capillary walls, or that they reach the blood circulation indirectly by first following the course of the lymphatics.

Toxemia results from the absorption of the poisonous bacterial products from non-invasive bacteria, as in tetanus.

THE CARDINAL CONDITIONS OF INFECTION.

Infection can take place only when the micro-organisms are sufficiently virulent, when they enter in sufficient number, when they enter by appropriate avenues, and when the host is susceptible to their action.

1. Virulence.—Virulence may be defined as the disease-producing power of micro-organisms. It depends upon the invasiveness of the bacteria or the toxicity of their products.

It is a variable quality of bacteria as they are found in the morbid conditions with which they are associated. It is a still more variable quality of most bacteria as they are cultivated in the laboratory. A few bacteria are almost constant in their pathogenic power, and cultures of Bacillus tuberculosis and B. mallei can be kept under artificial conditions for years with very little change. Some other bacteria begin to diminish in activity so soon as they are introduced to the artificial conditions of life in the test-tube, and in a few days, or weeks or months, as the case may be, lose their pathogenic power altogether or in large part. Still others, and perhaps the greater number, can be modified, and their pathogenic activity increased or diminished according to the experimental manipulations to which they are subjected.

Variation in virulence is not always a peculiarity of the species, for the greatest difference may be observed among individuals of the same kind. Thus the streptococcus usually attenuates rapidly when kept in artificial media, and various measures have been suggested for modifying and maintaining it, but Holst observed one culture whose virulence was unaltered after eight years' cultivation in the laboratory without any particular attention having been devoted to it.

Decrease of virulence under artificial conditions probably depends upon artificial selection of the organisms in transplantation from culture to culture. When planted upon artificial media, the vegetative members of the bacterial family proceed to grow actively and soon exceed in

number their less vegetative but more pathogenic fellows. Each time the culture is transplanted, more of the vegetative and fewer of the pathogenic forms are carried over, until after the organism is said to be accustomed to its new environment, and grows readily upon the artificial media, it is found that the pathogenic organisms have been largely or entirely eliminated and the vegetative forms alone remain.

Increase of virulence can rarely be effected without recourse to animal inoculations, or some modification of the culture method in which animal juices play an important part. It can be explained in the same way as decrease of virulence, by supposing that the method adopted for culture is adapted to the needs of the pathogenic members of the family and less so to the more distinctly vegetative forms.

Three means of increasing the virulence of bacteria are sufficiently important to merit the attention of the student.

1. *Passage through animals.* Except in cases where the virulence of the micro-organism is invariable, it is usually observed that the transplantation of the organism from animal to animal without intermediate culture *in vitro* greatly augments its pathogenic power. Of course, this artificially selects each time those members of the bacterial family best qualified for development in the animal body, eliminating the others, and the virulence correspondingly increases.

The increase in virulence thus brought about is, however, not so much an increase in the general pathogenic power of the organism for all animals, as in pathogenic activity toward the particular animal or kind of animal used in the experiments. Thus, in general, the passage of bacteria through mice increases their virulence for mice, but not necessarily for cats or horses; passage through rabbits, the virulence for rabbits, but not necessarily for dogs or pigeons, etc.

This specific character of the virulence is well accounted for by the "lateral-chain theory of immunity," where it will again be considered.

2. *The use of collodion sacs.* When cultures of bacteria are enclosed in collodion sacs and placed in the abdominal or other body cavities of animals, and kept in this manner through successive generations, the virulence is usually considerably increased. This is one of the favorite methods used by the French investigators. It keeps the bacteria in constant contact with the slightly modified body juices of

the animal, which transfuse through the collodion, and thus impedes the development of such organisms as are not able to endure their injurious influences. Thus it becomes only another way of carrying on an artificial selection of those members of the bacterial family that can endure, and eliminating those that cannot endure the defensive agencies of those juices with which the organisms come in contact.

3. *The addition of animal fluids to the culture-media* sometimes enables the investigator to increase, and usually enables him to maintain, the virulence of bacteria. In adding blood-serum, ascitic fluid, pleuritic exudate, etc., to bouillon, agar-agar, and other standard media, the body juices become too dilute for the same action to take place as in the "passage" or collodion sac, and this dilution of the constituents probably explains the slowness of the method in cases in which it succeeds, and the failures in cases in which it does not. Shaw* found that he could exalt the virulence of anthrax bacilli by cultivating them upon blood-serum agar for fourteen generations, after which they were three times as active as cultures similarly transferred upon ordinary agar-agar.

The increase under such conditions as these probably depends upon the immunization of the bacteria to the body juices of the animals, and this whole matter will be understood after the subject "Immunity" has been considered.

2. Number.—The number of bacteria entering the infected animal has a very important bearing upon infection, and may determine whether it shall occur or not.

It seems to be true of very few varieties of bacteria that the entrance of a single one may produce infection. In most cases a number of organisms are necessary in order that some may survive the sudden introduction to the new environment. Park points out that when bacteria are transplanted from culture to culture, many of them die. It seems not improbable, therefore, that when they are transplanted to an environment in which are present certain mechanisms for defending the organism against them, many more must inevitably die. The more virulent an organism is, the fewer will be the number required to infect. Marmorek, in his experiments with antistreptococcic serum, used a streptococcus whose virulence was exalted by passage through rabbits and intermediate cultivation upon agar-

* "Brit. Med. Jour.," May 9, 1903.

agar containing ascitic fluid, until one hundred thousand millionth of a cubic centimeter (*un cent milliardieme*) was fatal for a rabbit. In this quantity it is scarcely probable that more than a single coccus could have been present. Single anthrax or glanders bacilli may infect rabbits and guinea-pigs. Roger found that 820 tubercle bacilli from the culture with which he experimented were required to infect a guinea-pig, when introduced beneath the skin. Herman found that it required 4 or 5 c.c. of a culture of Staphylococcus pyogenes to produce suppuration in the peritoneal cavity of an animal; 0.75 c.c. to produce it beneath the skin; 0.25 c.c. in the pleura; 0.05 c.c. in the veins and 0.0001 c.c. in the anterior chamber of the eye.

In experimenting with Bacillus proteus vulgaris, Watson Cheyne found that 5,000,000 to 6,000,000 organisms injected beneath the skin did not produce any lesion; 8,000,000 caused the formation of an abscess; 56,000,000 produced a phlegmon from which the animal died in five or six weeks and 225,000,000 were required to cause the death of the animal in twenty-four hours. In studying Staphylococcus aureus upon rabbits he found that 25,000,000 would cause an abscess, but 1,000,000,000 were necessary to cause death.

3. The Avenue of Infection.—The successful invasion of the body by certain bacteria can be achieved only when they enter it through appropriate avenues. Even when invasion is possible through several channels, it is usually found that one of them, that by which the parasite most commonly invades the host, is most appropriate, and that invasion through it furnishes the typical picture of the infection.

Thus, gonococci usually reach the body through the urogenital mucous membranes, where they set up the various inflammatory reactions collectively known as gonorrhea—*i. e.*, urethritis, vaginitis, prostatitis, orchitis, cystitis, etc. These constitute the typical picture of infection. The organism may also successfully invade the conjunctiva, producing blennorrhea, but there is no evidence that gonococci can successfully invade the body through the skin or alimentary mucous membrane.

Typhoid and cholera infections seem to take place through the alimentary mucous membrane, and the evidence that infection takes place by inhalation is slight. It is not known to take place through the urogenital system, the conjunctiva, or the skin.

The avenue of entrance not only may determine that infection is possible, or impossible, but may also determine the form that it takes. This is well exemplified in tuberculosis. Tubercle bacilli rubbed into the deeper layer of the skin produce a chronic inflammatory disease, called *lupus*, that lasts for years and rarely results in generalized tuberculosis. Bacilli reaching the cervical or other lymph nodes by entrance through the tonsils, may remain localized, producing enlargement and softening of the nodes, or passing through them reach the circulation, in which they may be carried to the bones and joints and occasion chronic inflammation with necrosis and ultimate evacuation or exfoliation of the diseased mass, after which the patient may recover. Bacilli entering the intestine in many cases produce implantation lesions in the intestinal walls; bacilli inhaled into the lung, or conveyed to it from the intestine by the thoracic duct and veins, produce the ordinary pulmonary tuberculosis known as phthisis or consumption.

Pneumococci colonizing in the pharynx have been known to produce pseudomembranous angina; in the lungs, pneumonia; in the meninges, meningitis; in the cardiac valves, endocarditis; on the conjunctiva, conjunctivitis. In all of these cases we can look upon the type of infection as depending largely upon the portal through which the invading organism found its way into the tissues.

The avenue of entrance is, for obvious reasons, less important when the micro-organism is of the rapidly invasive form whose chief operation is in the streaming blood or in the lymphatics. Anthrax in most animals is characterized by a bacteremia regardless of the point of primary infection. Bubonic plague rapidly becomes a bacteremia regardless of the entrance of the Bacillus pestis by inhalation into the lungs, or by way of the lymphatics through superficial lesions.

It is not always possible to explain why certain channels should seem so much more appropriate for infection than others. When they are introduced beneath the skin, bacteria are, in most cases, delayed in reaching the circulation, and are in the meantime subjected to the action of the various defensive mechanisms prepared for their destruction. Many succumb to these after a time and never penetrate more deeply into the body. Others are transported to the lymph nodes, where they are destroyed, or, passing through these and succeeding in reaching the venous channels,

have next to pass through the pulmonary capillaries, where they are apt to be caught and destroyed without reaching the arterial system, by which they might be distributed to new fields of activity. The systemic circulation is similarly defended against such micro-organisms as reach the veins through lesions or accidents of the abdominal viscera, by the interposition of the portal capillary network of the liver, where the bacteria are caught and many of them destroyed, or passing through which, the pulmonary capillary system acts as a second barrier against them. The deeper the penetration, the more active the defense becomes, the blood itself furnishing agglutinins, bacteriolysins, and phagocytes for the protection of the host.

These defenses, however, are of no avail against actively invasive organisms which when injected directly into the streaming blood produce their effects more rapidly and in smaller numbers than when injected beneath the skin or elsewhere, because the field of operation is immediately reached instead of through a roundabout course. Taking anthrax bacilli, whose invasiveness has already been dwelt upon, as a typical illustration of this, Roger* found that when these organisms were injected into the aorta, the animals died much more quickly than when they were injected into the veins and were so obliged to find their way through the pulmonary capillaries to the general circulation. If the injections were made into the portal vein, they stood a good chance of recovery, the liver possessing the power of destroying sixty-four times as many anthrax bacilli as would prove fatal if introduced through other channels.

The defenses differ, however, in action upon different infectious agents, for when Roger modified the experiment by using streptococci instead of anthrax bacilli, he found that animals inoculated into the portal vein died more quickly than others injected into the aorta, and that animals injected into the peripheral veins lived longest, the liver apparently being far less destructive to streptococci than the lungs.

4. The Susceptibility of the Host.—Susceptibility is liability to infection. It is a condition in which the host is unable to defend itself against invading micro-organisms. Unusual or unnatural susceptibility is also spoken of as *predisposition.*

Many animals and plants are naturally without any means

* "Introduction to the Study of Medicine," p. 151.

of overcoming the invasiveness of certain parasitic micro-organisms, and are so naturally susceptible; others are normally able to resist their inroads, but because of various temporary or permanent physiological changes in their constitution, lose such power.

In general, it is true that any condition that depresses or diminishes the general physiological activity of an animal diminishes its ability to defend itself against the pathogenic action of bacteria, and so predisposes to disease. These changes are often so subtile that they escape detection, though at times they can be partly understood.

Depressing hygienic conditions of all kinds are shown by statistics to predispose to infectious diseases of all kinds. So many factors are combined in this broad statement, however, that it is difficult to subject them to a satisfactory analysis. Doubtless the expression stands for the sum of all the conditions that can be separately considered.

(*a*) *The inhalation of noxious vapors.* It has long been supposed that sewer gas was responsible for the occurrence of many infectious diseases, and when the nature of such diseases was made clear by a more complete knowledge of their bacterial causes, the fact still seemed to remain that defective sewage was in some way connected with the incidence of disease. It is difficult to prove its truth experimentally and the relation may be traditional and imaginary, though it is still commonly supposed that the vapors of sewage produce some depression of the vital powers. Men who work in sewers and plumbers who breathe much sewer gas are not apparently affected. Alessi* found that rats, rabbits, and guinea-pigs kept in cages some of which were placed over the opening of a privy, while in others the excreta of the animals were allowed to accumulate, suffered from a pronounced dimunition of the resisting powers. This would seem to be inconsistent with the habits of rats, many of which live in sewers. Abbott† caused rabbits to breathe air forced through sewage and putrid meat infusions for one hundred and twenty-nine days, and found that the products of decomposition inhaled by the animals played no part in producing disease, or in inducing susceptibility to it.

(*b*) *Fatigue* is a well-recognized clinical cause of susceptibility to disease, and experimental evidence of its

* "Centralbl. f. Bakt.," etc., 1894, xv, p. 228.
† "Trans. Assoc. Amer. Phys.," 1895.

correctness is not wanting. Charrin and Roger* found that white rats, which naturally resist infection with anthrax, succumbed to the infection if compelled to turn a revolving wheel until exhausted before inoculation.

(*c*) *Exposure* to cold seriously influences the resisting power of the warm-blooded animals. It is an everyday experience that chilling the body predisposes to "cold" and may be the starting-point of pneumonia. Pasteur found that fowls, which resist anthrax under normal conditions, succumbed to infection if kept for some time in a cold bath before inoculation.

The reverse seems to be true of the cold-blooded animals, for Gibier† found that frogs, naturally resistant to the anthrax bacillus, would succumb to infection if kept at 37° C. after inoculation.

(*d*) *Diet* produces some variation in the resisting powers. The tendency of scorbutics to suffer from infectious disorders of the mouth, the frequency with which epidemics of infectious disease follow famines, and the enterocolitis of marasmatic infants, illustrate the effects of insufficient food in predisposing to disease. We also find that the infectious diseases of carnivorous animals are not the same as those of herbivorous animals, and that the former are exempt from many disorders to which the latter quickly succumb. Hankin was able to show experimentally that meat-fed rats resisted anthrax infection far better than rats fed upon bread.

(*e*) *Intoxication* of all kinds predisposes to infection. Platania‡ found that such animals as frogs, pigeons, and dogs became susceptible to anthrax when under the influence of curare, chloral, and alcohol. Leo§ found that white rats fed upon phloridzin became susceptible to anthrax. Wagner‖ found that pigeons become susceptible to anthrax when under the influence of chloral. Abbott** found the resisting powers of rabbits against Streptococcus pyogenes and

* "Compte rendu Soc. de Biol de Paris," Jan. 24, 1890.

† "Compte rendu Acad. des Sciences de Paris," 1882, t. xcix, p. 1605.

‡ See Sternberg's "Immunity and Serum Therapy," p. 10; "Centralbl. f. Bakt.," etc., Bd. vii, p. 405.

§ "Zeitschrift für Hyg.," Bd. vii, p. 505, 1889.

‖ "Wratsch," 1890, 39, 40.

** "Jour. of Exp. Med.," vol. i, No. 3, 1896.

Bacillus coli diminished by daily intoxication with 5 to 15 c.c. of alcohol introduced into the stomach through a tube.

It is a common clinical observation that excessive indulgence in alcohol predisposes to certain infections, notably pneumonia, and every surgeon knows the danger of pneumonia after anesthetization with ether.

(*f*) *Traumatic injury* and mutilation of the body are not without effect upon infection. The more extensive the damage done to the tissues, the greater the danger of infection, and the more serious the consequences of infection when it takes place.

The mutilation of the body by the removal of certain organs is of disputed importance. There is much literature upon the effect of the spleen in overcoming infectious agents, but the experimental evidence seems about equally divided as to whether an animal is more or less susceptible after the removal of this organ than it was before.

(*g*) *Morbid conditions in general* predispose to infection. The frequency with which diabetics suffer from furuncles, carbuncles, and local gangrenous lesions of the skin; the increased susceptibility of phthisics to bronchopneumonia of other than tuberculous origin; the apparent predisposition of injured joints and pneumonic lungs to tuberculosis; the extensive streptococcus invasions accompanying scarlatina and variola; the presence of Bacillus icteroides and various other organisms in the blood and tissues of yellow fever patients, and the presence of Bacillus sui pestifer in the bodies of hogs suffering with hog cholera, all show the diminution in the general resisting power of an individual already diseased.

MIXED INFECTIONS.

The general prevalence of bacteria determines that few can enter and infect the body of a host without the association of other kinds. Therefore their operation in the body is subject to modifications produced in them or in the host by these associated organisms.

In experimental investigations this fact it not infrequently forgotten, and it is often remarked with surprise that the results of inoculation with pure cultures of a bacterium may be clinically different from those observed under natural conditions.

The tetanus bacillus, which endures with difficulty the

effects of uncombined oxygen, flourishes in association with saprophytic organisms by which the oxygen is absorbed. The same thing is probably true of other obligatory anaerobic organisms.

The metabolic products of one species may intensify or accelerate the action of those of an associated species, or the reverse may be true and the products of different organisms, having different chemical composition, may neutralize one another, or combine to form some entirely new substance which is entirely different from its antecedents. Such conditions cannot fail to influence the animal infected.

CHAPTER IV.

IMMUNITY.

IMMUNITY is ability to resist infection. It is the ability of an organism to successfully antagonize the invasive powers of bacteria, or to annul the injurious properties of their products. The mechanism of immunity is complicated or otherwise according to the necessity of the case. When the invasive action of non-toxicogenic bacteria is to be overcome, certain reactions, mostly on the part of the phagocytic cells, are called into action; when the toxic products of bacteria are to be deprived of injurious effects, the reaction seems to take place between the toxin and certain combining and neutralizing substances contained in the body juices; when bacterial invasion and intoxication are both to be antagonized, both mechanisms are engaged in the defenses. The reactions of immunity are, therefore, comparatively simple or exceedingly complex, according to the conditions involved, and the more involved these conditions become, the more complicated the defensive reactions become, until it may no longer be possible accurately to analyze them.

Some have endeavored to refer all of the phenomena of immunity to the ability of the animal to endure the bacteriotoxins, and have sought to relegate the reactions against invasion to a subsidiary place. This is undoubtedly an error, as the mechanisms are different and the prompt action of one may make the action of the other unnecessary. Metschnikoff* found that frogs injected with 0.5 c.c. of cholera toxin died promptly, but that frogs injected with cultures of the cholera spirillum recovered without illness. This would suggest that the recovery of the infected frog depended upon some defensive mechanism combating the invasiveness of the bacteria and so preventing the production of the toxin to which the frog was susceptible.

Immunity must not be conceived as something insepar-

* "Immunite dans les Maladies Infectieuses," Paris, 1901, p. 150.

ably associated with infection. The reactions of the body toward bacteria in the infectious diseases are identical with those toward other minute irritative bodies, and the reaction toward bacterio-toxins is identical with those toward other active substances, so that the only way by which a satisfactory understanding of the phenomena of immunity can be reached is by carefully comparing the reactions produced by bacteria and their products with those produced by other active bodies.

Immunity is called *active* when the animal protects itself through its own activities, *passive* when its protection depends upon defensive substances prepared by some other animal and forced upon it. Thus, if a frog be injected with anthrax bacilli, the leukocytes devour the bacteria, destroy them, and so protect the frog from infection, the immunity is active because it depends upon the activity of the frog's phagocytes. But if a guinea-pig previously given antitetanic serum be injected with tetanus toxin, and so recovers from the toxin, the resisting power, conferred by the antitoxin previously injected, does not depend upon any activity of the animal infected, which remains entirely passive.

Immunity is largely *relative.* Fowls are immune against tetanus, that is, they can endure, without injury, as much toxin as tetanus bacilli can produce in their bodies, and suffer no ill effects from inoculation. If, however, a large quantity of tetanotoxin produced in a test-tube be introduced into their bodies, they succumb to it. Mongooses and hedgehogs are immune against the venoms of serpents so as to be resistant to as much poison as is ordinarily injected by the serpents, but by collecting the venom from several serpents and injecting considerable quantities of it, both animals can be killed. Rats cannot be killed by injection with Bacillus diphtheriæ, and Cobbett* found that they could endure from 1500 to 1800 times as much diphtheria toxin as guinea-pigs, though more than this would kill them.

Carl Fraenkel has expressed the whole matter very tritely when he says: "A white rat is immune against anthrax in doses sufficiently large to kill a rabbit, but not necessarily against a dose sufficiently large to kill an elephant."

* "Brit. Med. Jour.," April 15, 1899.

NATURAL IMMUNITY.

Natural immunity is the natural, inherited resistance against infection or intoxication, peculiar to certain groups of animals, but common to all the individuals of those groups.

Few micro-organisms are capable of affecting all varieties of animals; indeed, it is doubtful whether any known organism possesses such universally invasive powers.

The micro-organisms of suppuration seem able to infect animals of many different kinds, sometimes producing local lesions, sometimes invading rapidly with resulting bacteremia. The tubercle bacillus is known to be pathogenic for mammals, birds, reptiles, batrachians, and fishes, though it is still uncertain whether the infecting organisms in these cases are identical or slightly differing species.

As a rule, however, the infectivity of bacteria and other micro-organisms is restricted to certain groups of animals which usually have more or less resemblance to one another; thus, anthrax is essentially a disease of warm-blooded animals, though certain exceptions are observed, and Metschnikoff has found that hippocampi (sea-horses), perch, crickets, and certain mussels are susceptible. Among the warm-blooded animals anthrax is most frequent among the herbivora, though some carnivora may also be infected.

Close relationship is not, however, a guarantee that animals will behave similarly toward infection. The rabbit, guinea-pig, and the rat are rodents, but though the rabbit and guinea-pig are susceptible to anthrax, the rat is immune. This is still better exemplified in the susceptibility of mice to glanders. The field-mouse seems to be the most susceptible of all animals to infection with Bacillus mallei; the house mouse is much less susceptible, and the white mouse is immune. Mosquitos, though closely related, are different in their immunity to the malarial parasite. The culex does not harbor the parasite at all, and of the anopheles, two very similar species seem to behave very differently, Anopheles maculipennis being the common definitive host of the parasite, while Anopheles punctipennis is not known to be susceptible to it. The same differences appear to exist among the members of the human species. It is asserted that Mongolians, and especially Japanese, are immune against scarlatina, and that negroes are immune against

yellow fever, though not all the facts are definitely known regarding these usually cited examples.

Human beings suffer from typhoid, cholera, measles, scarlatina, yellow fever, Malta fever, and numerous other diseases unknown among the lower animals, even those domestic animals with which they come in close contact. They also suffer from anthrax, rabies, glanders, bubonic plague, and tuberculosis, which are common among the lower animals. Animals in turn suffer from distemper, hog cholera, Texas fever, swine-plague, chicken cholera, mouse septicemia, etc., the respective micro-organisms of which are not known to infect man.

It has already been pointed out that mongooses and hedgehogs are immune against the venom of serpents from which other animals quickly die. The tobacco worm lives solely upon tobacco-leaves, the juice of which is intensely poisonous to higher animals, and is also a good insecticide. Boxed cigars and baled tobacco are often ruined by the larvæ of a small beetle that feeds upon them, and a glance over the poisonous vegetables will show that few of them escape the attacks of insects immune against their active juices.

This collection of facts is sufficient to show that many animals are by nature immune against the invasion of microparasites of certain kinds, and that they are also at times immune against poisons. Immunity against one kind of infection or intoxication is, however, entirely independent of all other infections and intoxications. Immunity against infection usually guarantees exemption from the toxic products of that particular micro-organism, though experiment may show the animal to be susceptible to it. Immunity against any form of bacterio-toxin usually, though not necessarily, determines that the micro-organism, though it may be able to invade the body, can do very little harm.

ACQUIRED IMMUNITY.

Acquired immunity is resistance against infection or intoxication possessed by certain animals, of a naturally susceptible kind, in consequence of conditions peculiar to them as individuals. It is a peculiarity of the individual, not of his kind, and signifies a subtile change in physiology by which latent defensive powers are stimulated to action.

The reactions in general correspond with those of natural immunity, and comprise mechanisms for overcoming the invasion of pathogenic organisms, for neutralizing or destroying their toxins or both. As an acquired character and an individual peculiarity it is not transmitted to the offspring, though these sometimes also acquire immunity through the parents. Thus in studying immunity of mice against ricin, Ehrlich found that the newly born offspring of an immune mother were not immune, though they subsequently became so through the mother's milk.

Acquired immunity differs from natural immunity in being less certain and of variable duration. The animal may be immune to-day, but lose all power of defending itself a month hence.

Natural immunity is always active, but certain forms of acquired immunity are passive.

Immunity may be acquired through *infection* or *intoxication*, and either of these may be accidental or experimental.

(A) **Active Acquired Immunity.**—**1. Immunity Acquired through Infection.**—(*a*) *Accidental Infection.*—The most familiar form of acquired immunity follows an attack of an infectious disease. Every one knows that an attack of measles, scarlatina, varicella, variola, yellow fever, typhoid fever, and other common infectious maladies, is a fairly good guarantee of future exemption from the respective disease. Immunity thus acquired is not transmissible to the offspring. Almost everybody has had measles, yet almost all children are born susceptible to it. It is not necessarily permanent, as is shown by the not infrequent cases in which second attacks of measles occur. In some cases, as after typhoid fever, the immunity is not at first observable and the patient may suffer from relapses. Later it becomes well-established and no repetition of the disease is possible for years.

Sometimes the infection, by which immunity is acquired, is not exactly similar to the disease against which it affords protection, as in the case of varicella, which protects against variola. It is still controversial, however, whether cow-pox is variola of the cow, or an entirely different disease. Cow-pox was, however, much more common in the days when smallpox was common, and has now become an extremely rare disease.

(*b*) *Experimental Infection.*—1. *Inoculation:* The oldest experiments in immunity date from unknown antiquity and were practised in China and other Oriental countries for the purpose of preventing smallpox. The Chinese method of experimentally producing variolous infection was very crude and consisted in the introduction of the crusts from cases of variola into the nose, and tying them upon the skin. The Turkish method was much more neat, in that a small quantity of the variolous pus was introduced into a scarification upon the skin of the individual to be protected. Lady Montague, wife of the British Ambassador, brought the so-called "inoculation" method of preventing smallpox from Turkey in the early part of the eighteenth century (1718). By both methods the very disease, variola, against which protection was desired, was occasioned, the only advantage of the experimental over the accidental infection being that by selecting the infective virus from a mild case of variola, by performing the operation at a time when no epidemic of the disease was raging, and by doing it at a time when the person infected was in the most perfect physical condition, the dangers of the malady might be mitigated.

The danger always existed, however, that the induced disease being true variola might prove unexpectedly severe, and that each inoculated individual, suffering from the contagious disease, might start an epidemic.

2. *Vaccination:* In 1791 a country schoolmaster named Plett, living in the town of Starkendorf near Kiel in Germany, seems to have made the first endeavor to subject to experimental demonstration the oft-repeated observation that persons who had acquired cow-pox did not subsequently become infected with smallpox, by inserting cow-pox virus into three children, all of whom escaped smallpox.

The father of vaccination, and the man to whom the world owes one of its greatest debts, was Edward Jenner, who performed his first experiment on May 14, 1796, when he transferred some of the contents of a cow-pox pustule on the arm of a milkmaid named Sarah Nelmess to the arm of a boy named John Phips. After the lad had recovered from the experimental cow-pox thus produced, he subsequently introduced smallpox pus into his arm and found him fully immunized and rendered insusceptible to the disease. This led Jenner to perform many other experiments, and record his observations in numerous scientific memoirs. The

success of his work immediately attracted the attention of both scientific investigators and sanitarians, and its outcome has been the establishment of compulsory vaccination by legal enactment in nearly all civilized countries, with the result that smallpox, instead of being one of the most prevalent and most dreaded diseases, has become one of the most rare and least feared.

The immunity acquired through vaccination is active and usually of prolonged duration. It is subject to the same variations observed in other experimentally acquired immunities, these variations explaining the occasional failures which constitute the "stock in trade" of those who still remain unconvinced of the scientific basis and efficacy of the procedure.

Though a thorough analysis of the irregularities and exceptions of vaccination would be of much interest, a brief mention of the most important must suffice for the present argument.

The first controversial point is the nature of the "vaccine," or virus used in the operation. It is obtained from calves or heifers suffering from experimental cow-pox, and is a virus descended from various spontaneous cases of cow-pox observed in places remote from one another. Experts are undecided whether cow-pox is variola modified by passage through the cow so that the transplanted micro-organisms are only capable of inducing a local instead of a general disease, or whether it is an independent affection natural to the cow.

In reality the matter in unimportant, so long as the desired effect is accomplished, and the true lineage of the virus is only a matter of scientific curiosity. As immunity is almost invariably a specific effect resulting from infection, it would seem most likely that cow-pox and smallpox were originally identical.

The advantage of "vaccination" over "inoculation" is that the induced disease is local and not dangerous except in rare cases, and that it is not contagious. The natural variations in the susceptibility of different vaccinated individuals determine that a few persons cannot be successfully vaccinated, being immune to the mildly invasive organisms of vaccinia, though perhaps susceptible to the actively invasive organisms of variola; that a few individuals shall prove abnormally susceptible to vaccinia so that the disease

departs from its usual local type and generalizes, but that in nearly all cases the disease will follow the well-known type of a local lesion characterized by definite periods of incubation, vesiculation, pustulation, and cicatrization.

The occasional variations in immunity of different individuals also determine that having been vaccinated once an individual may not again become susceptible to vaccination, though he may become susceptible to the more actively invasive organisms of variola, or that he may soon become again susceptible to both diseases, or that in very rare cases no immunity against variola will result from vaccination. In most cases successful vaccination can be repeated once or twice at intervals of seven or ten years, and experience shows that the immunity against smallpox conferred by vaccination is of longer duration and usually becomes permanent after vaccination has been repeated once or twice.

Sanitarians are accustomed to speak of efficient and inefficient vaccination. These are vague terms and do not seem to be understood by the laity. Efficient vaccination is vaccination repeated as often as is necessary. It has already been shown that individual variations determine that a few individuals never become immune, hence never can be efficiently vaccinated. Other persons are efficiently vaccinated by a single operation. The term is usually interpreted to indicate that which experience has shown to be efficient in average cases.

Failures not uncommonly result from causes having nothing to do with the problems of immunity. The operation is not the essential, but only the means of introducing the virus by which the disease is induced. That an operation of scarification has been performed upon a child, and that a scar has remained thereafter may mean nothing. It is not the *operation* but the *disease* that achieves the result, and if the operation be improperly done, poor—*i. e.*, old or inert—matter introduced, or if after introduction it be destroyed by the application of antiseptics, no effect can be expected. Hence all persons that have been vaccinated may not have had vaccinia, the essential condition leading to immunity. Nor does the occurrence of a local lesion act as a guarantee that vaccinia has been induced. Careful examination of the resulting lesions should always be made, that the type of the infection may be studied. It is the *disease, vaccinia*, that must occur—three days incubation,

three days vesiculation, three days pustulation, and subsequent cicatrization with the formation of a punctate scar.

An arm may be never so sore, may suppurate or even become gangrenous, without vaccinia having occurred or the desired benefit attained.

The accidents of vaccination were formerly numerous and sometimes disastrous because of the general inattention to the quality of the materials used, the mode of inserting them, the condition of the patient's skin, and the careless treatment of the resulting lesions. When human virus was used, the transmission of human diseases, such as syphilis and erysipelas, occasionally took place; now these are rare accidents indeed. When no attention was paid to the quality of the bovine virus, and no governmental inspection of laboratories required, the accidental contamination of the virus occasioned a small number of accidental infections of the patients' arms, but these evils are becoming less and less as greater attention is given to the details of the process. Some accidents and some few deaths there will probably always be, just as there are occasional accidents and occasional fatal results following all kinds of trivial injuries, though care will eliminate them as the sources of accident are better understood.

3. *Bacterination:* Although the word vaccination is derived from the Latin *vacca*, "a cow," and was first employed in connection with Jenner's method of introducing virus modified by passage through a cow, it has now by custom become applicable to any kind of protective inoculation, and the word *bacterination* is only introduced for the purpose of indicating certain differences in the method.

In 1881 Pasteur,* in experimenting with Bacillus anthracis, observed that if the organism were cultivated at unusually high temperatures it lost the power of producing spores, and diminished in virulence. He also found that when the organisms had been so attenuated, they could not regain virulence without artificial manipulation. It occurred to him that such organisms, possessing feeble virulence, might be able to confer immunity upon animals into which they were inoculated, and continued to investigate the subject until he found that by using three "vaccines" or modified cultures of increasing virulence, it was possible to render animals immune

* "Compte rendu de la Soc. de Biol de Paris," 1881, XCII, pp. 662–665.

against the unmodified organisms. (See Anthrax.) This method was put to practical test with great success, and has since been extensively practised in different parts of the world.

Arloing, Cornevin and Thomas,* and Kitt† found that exposure of the bacilli to a high temperature in the dry state modified the virulence of the Bacillus anthracis symptomatici, and devised a practical method of protecting cattle against symptomatic anthrax by inoculating them with powdered muscle tissue containing the bacilli attenuated by drying and exposure to 85° C. This method (see Symptomatic Anthrax) has been in use in many countries with great success ever since, and has given excellent satisfaction.

In 1889 Pasteur,‡ continuing his researches upon the experimental modification of the germs of disease and their use as prophylactics published his famous work upon rabies, and showed that, although the micro-organism of that disease had so far eluded discovery, it was contained in the central nervous system of diseased animals, where it could be modified in virulence by drying. By placing spinal cords removed from rabid rabbits in a glass jar containing calcium chlorid, he was able to diminish the virulence of the contained micro-organisms according to the duration of the exposure. The introduction of the attenuated virus was followed by the development of a certain degree of immunity, and was then followed by a more active virus, until by repeated treatments the animals acquired complete immunity against street virus. (See Rabies or Hydrophobia.) These experiments formed the basis of the "Pasteur method" of treating rabies, which is nothing more than immunization during the incubation period with the modified germs of the disease.

Haffkine§ found that the introduction of killed cultures of virulent cholera spirilla produced immunity against the living micro-organisms, and used the method with considerable success for preventing the disease. Later‖ he applied the same method, also with considerable success, for the prevention of bubonic plague.

* "Le Charbon Symptomatique du Bœuf," Paris, 1887.
† "Centralbl. f. Bakt.," etc., I, p. 684.
‡ "Compte rendu de la Soc. de Biol. de Paris," 1881, CVIII, p. 1228.
§ "Brit. Med. Jour.," 1891, II, p. 1278.
‖ "Brit. Med. Jour.," 1895, II, p. 1541.

Wright* followed pretty much the same method for the prevention of typhoid fever and thought that much success was achieved, though others were not convinced of its benefit.

In all these cases the immunity induced by the experimental manipulations was specific in nature, and variable in intensity according to the method of treatment adopted and thoroughness with which it was carried out. This variability in the results attained will be much better understood after the subject of immunization against toxins has been discussed.

2. **Immunity Acquired by Intoxication.**—Bacterio-toxins form a miscellaneous group of active bodies of entirely different chemical composition and physiological activity. Some are toxalbumins, some are enzymes, some are bacterio-proteids. The true nature of the greater number of these bodies is unknown, but study of their physiological action has brought forth the important fact that their behavior toward the body cells is in no way different from the behavior of the same cells toward other chemical compounds of similar constitution, and that nearly all physiologically active bodies introduced into living bodies produce definite, though not necessarily visible reactions.

Some of the facts that bear upon immunity have long been known though only recently correlated with it. Thus from remote antiquity it has been known that those who regularly consumed small quantities of poisons become irresponsive to their action, and it is well known that Mithridates adopted this mode of defending himself from his enemies. The immunity of opium and arsenic eaters, and the pleasurable instead of poisonous effects of tobacco, are too well known to require further mention.

It was, however, only by beginning with the metabolic products of bacteria, studying their activities, and comparing them with other toxic and active substances that the present state of knowledge has been attained.

The work seems to have been begun by Chauveau,† who believed that the immunity conferred by bacteria was due to the presence of their soluble products, but the first direct demonstration was given by Salmon and Smith,‡ who, as

* "Brit. Med. Jour.," Jan. 30, 1897, p. 256.

† "Ann. de l'Inst. Pasteur," 1888, 2.

‡ "Centralbl. f. Bakt.," etc., II, No. 18.

early as 1886, showed that it was possible to immunize pigeons against hog-cholera by means of repeated injection with cultures exposed to 60° C., and containing no living organisms. Charrin* found it possible to immunize rabbits against Bacillus pyocyaneus by injecting them with the filtered products of cultures of that organism, and Bonome† similarly to immunize animals against Bacillus proteus, B. cholera gallinarum and the pneumococcus. Roux and Chamberland‡ and Roux § were able by the use of boiled cultures of the bacilli of malignant edema, and of quarter evil, similarly to immunize animals against these respective infections. These early experiments opened a wide field of investigation, which was subsequently continued by many others until it was found that the dead as well as the living microorganisms, and the products as well as the dead bacteria of most of the infectious diseases when properly introduced into animals, could occasion increased resisting power.

The subject was much further elaborated by Roux and Yersin‖ in their experiments with diphtheria toxin, by Behring** in his early studies of diphtheria, and by Kitasato†† in his experiments with tetanus.

(B) **Passive Acquired Immunity.**—Passive immunity is always acquired, never natural. It depends upon defensive factors not originating in the animal protected, but artificially or experimentally supplied to it. The fundamental principle is simple and has become the basis of serum therapeutics. If the immunized animal generates factors by which the infecting bacteria can be destroyed or the activity of their products overcome, why cannot these factors be removed from that animal and transferred to another?

The first experiments seem in this direction to have been made by Babes and Lepp,‡‡ who found that the blood-serum of animals immunized to rabies possessed a defensive power when injected into other animals. Ogata and Jasuhara§§ found that the subcutaneous injection of

* "Compte rendu," cv, p. 756.
† "Zeitschrift f. Hyg.," v, p. 415.
‡ "Ann. de l'Inst. Pasteur," 1887, 12. § Ibid., 1888, 2.
‖ "Ann. de l'Inst. Pasteur," II, 1888, p. 629.
** "Deutsche med. Wochenschrift," 1890, No. 50.
†† "Zeitschrift für Hygiene," x, 1891, p. 267.
‡‡ "Annales de l'Inst. Pasteur," 1889, vol. III.
§§ "Centralbl. f. Bakt.," etc., IX, p. 25, 1890.

blood-serum from an animal immunized against anthrax enabled the injected animals successfully to resist infection. Behring and Kitasato* found that the blood-serums of animals immunized against diphtheria and tetanus, when mixed with cultures of these respective bacilli, neutralized their power to produce disease. Kitasato† found that if mice were inoculated with tetanus bacilli, they could be saved from the fatal infection by the intra-abdominal injection of some blood-serum from a mouse immunized against tetanus, even after symptoms of the disease had appeared. Ehrlich‡ showed that the blood-serums of animals immunized against abrin and ricin could save other animals from the fatal effects of these respective alkaloids. Phisalix and Bertrand,§ and later Calmette,‖ found the blood-serum of animals immunized against the venoms of serpents, similarly possessed the power of neutralizing the poisonous effects of the venoms. Kossel** found that the blood-serum of animals immunized against the poisonous blood-serum of eels, contained a body which destroyed or neutralized the effects of the eels' serum.

Thus, it is shown that in each case in which defensive reactions are stimulated in experiment animals, these reactions are accompanied by the appearance in the blood-serum of those animals of factors that can be utilized to defend other animals in whose bodies no similar reactions have been produced.

When the defensive action departs from the simple type, and is complicated by the necessary association of immune and complementary bodies, the difficulty of producing passive immunity increases because two factors must be simultaneously present in the serum used. Thus, to enable an animal to destroy or dissolve an infecting micro-organism by which it is invaded and which its own defensive mechanisms cannot overcome, it is insufficient to inject the serum of an animal immunized against that bacterium, because such serum contains only an increased quantity of the immune or intermediate body, and no increase in the complement. The

* "Deutsche med. Wochenschrift," 1890, No. 49.
† "Zeitschrift für Hygiene," 1892, XII, p. 256.
‡ "Deutsche med. Wochenschrift," 1891, Nos. 32 and 44.
§ "Compte rendu Acad. des Sciences de Paris," CXVIII, p. 556.
‖ "Ann. de l'Inst. Pasteur," 1894, VIII, p. 275.
** "Berliner klin. Woch.," 1898, p. 152.

animal may need no additional immune body, or, receiving it, may be unable to use it because of deficient complement, and under such circumstances may even be injured by it, through the formation of anti-immune body by which complementary substance subsequently formed may be rendered inactive because of the neutralized immune body present in its blood. It is, therefore, doubtful whether an immune serum will be useful in assisting the animal—*i. e.*, producing passive immunity—in these cases.

On the other hand, the administration of a serum containing complementary substance is made difficult because of our inability experimentally to increase the complements normal to the blood. The administration of normal fresh serum sometimes supplies enough complement to enable the dissolution of bacteria to take place, but the quantity is usually so small that no appreciable benefit obtains.

Passive immunity may also be brought about in a few cases by the injection into the intoxicated animal of substances, other than immunity products, that have a specific affinity for the poison. Thus Wassermann and Takaki* found that when the crushed spinal cord of a rabbit was mixed *in vitro* with tetanus toxin, the poison was quickly absorbed by the nerve-cells, so that the mixture became inert and could be injected into animals without harm. Wassermann also found that the same effects could be produced in the bodies of animals, and that when the crushed spinal cord was injected into an animal twenty-four hours, or a few hours previously, or a few hours after a fatal dose of tetanus toxin, enough of the combining elements remained in the blood to fix the toxin before it anchored itself to the central nervous system of the intoxicated animal. Myers† found that the ground-up tissue of the adrenal bodies was able to fix and thus annul the poisonous effects of cobra venom *in vitro*.

In all these cases the neutralizing effects are either accomplished or initiated by factors prepared experimentally, and forced upon the animal in whose body their activities are manifested.

* "Berliner klin. Wochenschrift," Jan. 3, 1898.

† "Lancet," July 2, 1898.

SYNOPSIS OF THE EXPERIMENTAL STUDIES OF IMMUNITY.

The first comparative studies were made by Ehrlich,* in his work upon the vegetable alkaloids ricin, abrin, and robin. Kossel† investigated the reactions produced with toxic eels' blood and found that immunity could be established against their hemolytic action. Phisalix and Bertrand‡ showed that immunity could also be produced in guinea-pigs against the action of viper venom.

The investigations upon other active bodies were soon begun. In 1893 Hildebrand§ studied emulsin and found that it produced a definite reaction with the formation, in animals injected, of an anti-emulsin. v. Düngern‖ studied proteolytic enzymes of various bacteria, and showed that when gelatin-dissolving enzymes were repeatedly injected into animals, definite reactions took place, and in the serum a body appeared that inhibited the action of the ferment in a test-tube. Gheorghiewski** immunized animals to cultures of Bacillus pyocyaneus, and found that the reaction provoked caused the appearance in the serum of some body that prevented the formation of the blue pigment so characteristic of the organism. Morgenroth†† applied the same principle to rennet, finding that it produced definite reactions, with the formation of an anti-body inhibiting the coagulation of milk. Bordet and Gengou‡‡ found that the fibrin ferment of the blood of one animal was active in the body of another animal, producing an inhibiting substance by which the coagulation of the blood of the first animal could be delayed.

The studies of Kraus§§ showed a new fact, that when filtered cultures of the cholera spirillum were introduced into animals, the serum of these animals, added to the filtered culture in a test-tube, caused the appearance of a delicate flocculent precipitate.

* "Deutsche med. Woch.," 1891, Nos. 32 and 44.
† "Berliner klin. Wochenschrift," 1898.
‡ Atti d XI Congr. med. internaz. Roma, 1894, II, 200–202.
§ "Virchow's Archives," Bd. CXXXI.
‖ "Münchener med. Woch.," Aug. 15, 1898.
** "Ann. de l'Inst. Pasteur," 1899.
†† "Centralbl. f. Bakt.," etc., 1899, XXVI, p. 349
‡‡ "Ann. de l'Inst. Past.," 1903, XVII, p. 822.
§§ "Wien. klin. Woch.," 1897.

Wassermann and Schütze* found that when cows' milk was repeatedly injected into rabbits, their serum acquired the property of occasioning a precipitate when added to cows' milk, but not when added to goats' or any other milk. If, however, the rabbit had been repeatedly injected with goats' milk or human milk, its serum would precipitate with those milks, respectively, and not with cows' milk. The reaction was thus shown to be specific.

Meyers† found that the repeated intraperitoneal injection of egg-albumen into rabbits caused their serum to give a dense precipitate when added to crystallized egg-albumen.

Tchistowitch‡ found that eels' serum injected into animals produced a reaction in which immunity to its poisonous action was associated with the ability of their serum to produce a precipitate when added to the eels' serum.

Closely connected with these various reactions are certain others variously spoken of as cytotoxic, cytolytic, hemolytic, bacteriolytic, etc. The first observation bearing upon these was made by R. Pfeiffer,§ who found that when guinea-pigs received frequent intraperitoneal injections of cholera spirilla and became thoroughly immunized, their serum behaved very peculiarly toward the bacteria in the peritoneal cavity of freshly infected animals, in that it caused them to become aggregated into granular masses and subsequently to disappear. This became known as "Pfeiffer's phenomenon." The serum of the immunized animal was devoid of action by itself, the serum of the infected animal was inactive, but the combination of the two brought about dissolution of the micro-organisms. Later it was shown by Metschnikoff‖ that the living animal was not a factor in the process, but that what was seen in the peritoneal cavity could be reproduced in a test-tube, though not quite as well.

Bordet** made frequent injections of defibrinated rabbits' blood into guinea-pigs, and obtained a serum that had a solvent action upon the rabbit's corpuscles *in vitro*, and showed that the induced hemolysis resembled in all points the bacteriolysis.

* "Deutsche med. Woch.," 1900.
† "Lancet," II, 1900.
‡ "Ann. de l'Inst. Pasteur," vol. 13.
§ "Zeitschrift für Hygiene," XVIII.
‖ "Ann. de l'Inst. Pasteur," 1895.
** "Ann. de l'Inst. Pasteur," XII.

Ehrlich and Morgenroth* studied the hemolytic action of the serum of goats that had been frequently injected with the defibrinated blood of sheep and goats, and were able to point out the mechanism of the corpuscle solution or hemolysis. It was found to depend upon two associated factors, one of which, the lysin or solvent, was present in normal blood, and was called "addiment" or "complement," and another present only in the serum of the reactive animals, called the "immune body" or "intermediate body." The former was labile and easily destroyed by heat, the latter stabile and not affected by heat up to the point of coagulation. The experiments were confirmed by von Düngern and many others. It is to be observed in passing that this reaction differs from the direct solution of the corpuscles *in vitro* by cobralysin, which was studied by Myers,† and tetanolysin, studied by Madsen,‡ in that it is intermediate, and only brought about by the cooperation of two factors, while the action of the lysins of venom, the tetanus bacillus, the streptococcus, Bacillus pyocyaneus, and other micro-organisms, is direct and immediate.

Myers found, however, that the hemolytic substance of venom, and Madsen that the hemolytic products of Bacillus tetani, also produce reactions in animals, and that when successful immunization against them was accomplished, the serums of the experiment animals became antidotal or inhibiting to the action of the respective lysins.

Von Düngern§ found that by injecting dissociated epithelial cells from the trachea of oxen into the peritoneal cavity of guinea-pigs, it was possible to produce epitheliolysins; Lindemann,‖ that emulsions of kidney substance injected into animals caused them to form nephro-lysins or nephrotoxins; Landsteiner** and Metschnikoff†† in the same manner successfully prepared spermotoxin by injecting the spermatozoa of one animal into the peritoneal cavity of another. Metalnikoff‡‡ found that if he introduced the spermatozoa of

* "Berliner klin. Wochenscrift," 1899.
† "Trans. Path. Soc. of London," LI.
‡ "Zeitschr. f. Hyg.," 1899, XXXIII, p. 239.
§ "Münchener med. Wochenschrift," 1899.
‖ "Ann. de l'Inst. Pasteur," 1900.
** "Centralbl. f. Bakt.," etc., 1899, XXV.
†† "Ann. de l'Inst. Pasteur," 1899.
‡‡ "Ann. de l'Inst. Pasteur," 1900.

a guinea-pig into the peritoneum of another, the spermotoxic serum produced was solvent for the spermatozoa of both. Both Metschnikoff and Metalnikoff also found that the spermotoxin when introduced into animals was active in producing anti-spermotoxin by which the destructive action of the serum upon spermatozoa could be inhibited.

Metschnikoff* and Funck† found that animals treated with emulsions of the spleen, and mesenteric lymph nodes of one kind of animal, produced sera whose action was agglutinative and solvent for leukocytes and lymph cells. Delezene‡ found that dissociated liver cells injected into animals similarly caused the formation of a cytotoxic serum whose specific destructive action was upon them.

All of these reactions are indirect and intermediate, and take place under appropriate conditions both in the bodies of animals and in the test-tube.

But the number of reactions that can be brought about by physiologically and chemically active bodies introduced into the bodies of animals seems to be limitless, for Ehrlich and Morgenroth in their studies of hemolysis found that serums rich in "complement" produced reactions productive of anti-complements, and that serums rich in immune bodies, produced similar reactions yielding anti-immune bodies, and that these serums inhibited the activities of the respective factors by whose stimulation they were produced.

During the activity of these investigations, prompted by the results obtained in studying the infectious diseases, the bacteria were at no time forgotten, and as promptly as a new fact was determined, it was utilized to make clear some of the obscure problems of infection and immunity against infection.

The observation that bacteria subjected to the action of blood-serum in a test-tube were diminished in virulence or killed, made as early as 1884 by Grohman,§ von Fodor,‖ carefully studied by Nuttall,** and referred by Buchner††

* "Ann. de l'Inst. Pasteur," 1899.

† "Centralbl. f. Bakt.," etc., XXVII, 1900.

‡ "Compte rendu de l'Acad. des Sciences," 1900, CXXX, pp. 938, 1488.

§ "Untersuchungen aus dem physiol. Institut zu Dorpat," Dorpat, 1884, Krüger.

‖ "Centralbl. f. Bakt.," etc., 1890, VII, p. 753.

** "Zeitschrift für Hygiene," 1888, IV, p. 353.

†† "Centralbl. f. Bakt.," etc., V, p. 817; VI, p. 1; 561; XII, No. 24; "Berliner klin. Wochenschrift," 1892, p. 449; "Münchener med. Wochenschrift," 1892, Nos. 8 and 52; 1894, Nos. 24 and 25, pp. 717 and 744.

to solvents of a ferment nature to which he applied the name *alexin*, were many times repeated and confirmed. Pfeiffer and Kolle* showed that the bacteriolytic quality of the serum was intensified in experimental immunization, and Bordet† showed that the processes of hemolysis and bacteriolysis were identical so far as the factors engaged were concerned. "Pfeiffer's phenomenon" became susceptible of easy interpretation, and many facts of immunity formerly obscure were cleared up.

EXPLANATION OF IMMUNITY.

Before the facts now at our disposal had been gathered together, and before the phenomena of immunity against infection had been compared with those of intoxication, Pasteur‡ and Klebs§ endeavored to explain acquired immunity by supposing that micro-organisms living in the infected animal used up some substance essential to their existence, and so died out, leaving the soil unfit for further occupation. This hypothesis was known as the "exhaustion theory." Wernich‖ and Chauveau** thought it more probable that the micro-organisms after having lived in the body left behind them some substance inimical to their further existence. This hypothesis was known as the "retention theory." Neither of these hypotheses can be given more than a passing mention at present, as they both fail to explain natural immunity or immunity against intoxication.

Carl Roser†† observed that the leukocytes of the bodies of higher animals sometimes enclosed bacteria in their cytoplasm. Koch, Sternberg, and others confirmed the observation, but no attention was paid to it until Metschnikoff‡‡ correlated it with other known facts and original observations, and came to the conclusion that the enclosed bacteria had been eaten by the leukocytes in which they were killed

* "Centralbl. f. Bakt.," etc., xx.

† "Ann. de l'Inst. Pasteur," xii.

‡ "Compte rendu de la Soc. de Biol. de Paris," xci.

§ "Arch. f. experimentele Path. u. Pharmak.," xiii.

‖ "Virchow's Archives," Bd. lxxviii.

** "Compte rendu de la Soc. de Biol. de Paris," xc and xci.

†† "Biologie niederste Organismen," Marburg, 1881

‡‡ "Virchow's Archives," Bd. xcvi, p. 177; "Ann. de l'Inst. Pasteur," t. i, p. 321, 1887.

and digested, and that the behavior of the cells toward the bacteria afforded an explanation of the mechanism by which recovery from the infectious diseases takes place. According to the original interpretation upon which this, the "theory of phagocytosis" was founded, recovery in many if not all of the infectious diseases depends upon the successful destruction of the invading bacteria by the body cells, especially the leukocytes. These devouring cells Metschnikoff called *phagocytes*, and of them he recognized two classes, the *microphages*, which are white blood-corpuscles, and the *macrophages*, which are larger cells derived from the endothelial and other tissues. Metschnikoff, his associates, and his pupils soon collected evidence sufficient to show that phagocytosis, if not the chief factor in defending the body from infectious organisms, is at least an important one. Many of the most interesting facts are described in Metschnikoff's books, "Etudes sur l'Inflammation" and "Immunite dans les Maladies Infectieuses," which every interested student of the subject should read.

These studies show that in nearly all cases in which animals are naturally immune against infection, the leukocytes are active in their phagocytic behavior toward them; that in acquired immunity, the leukocytes previously inactive, become active toward them; that the enclosure of bacteria within the cells sometimes results in the death of the cells, sometimes in the death of the bacteria; that phagocytosis is much more active in diseases in which the bacteria have limited toxicogenic powers, and in which they probably exert a positively chemotactic influence upon the cells, than in cases in which the bacteria are strongly toxicogenic and probably exert an injurious and negatively chemotactic influence upon them, and that when the toxicogenic power of the bacteria is great, many of the phagocytes are killed and dissolved—*phagolysis*. Study of the primitive forms of animal life shows that amebæ constantly feed upon smaller organisms, some almost exclusively upon bacteria, which they are able to kill and digest through an intracellular enzyme demonstrated by Mouton,* and called *amebadiastase*, and regarded as a form of trypsin. The intracellular digestion of cœlenterate animals is accomplished by means of *actinodiastase*, an enzyme discovered by

*"Compte rendu de l'Acad des Sciences de Paris," 1901, CXXXIII, p. 244.

Fredericq, and studied by Mesnil. It seems to be related to papine and digests albuminoids. The digestion of erythrocytes and tissue fragments is accomplished through an enzyme of the macrophages, which Metschnikoff calls *macrocytase*, that of bacteria through an enzyme of the microphages, which he calls *microcytase*. In phagolysis these respective ferments are liberated into the plasma, imparting to it a bactericidal and bacteriolytic action similar to that normally peculiar to the cytoplasm of the cells. The dissemination of the enzymes in phagolysis, with resulting bacteriolytic power of the blood plasma and serum, is a later modification of the original conception of Metschnikoff, that the invading parasites were eaten up by the phagocytes, and was made necessary by the investigation of the bactericidal property of the body juices, to which some attention must now be devoted. The experiments of Wright and Douglas* indicate that the action of the phagocytes upon the bacteria is not immediate, but only subsequent to a preparative action upon the organisms by substances contained in serum, and to which they give the name "*Opsonins*" (Lat. *opsono*, "I prepare a meal for").

Long before Metschnikoff began his studies of the phagocytes Traube and Gscheidel† observed that the blood plasma possessed the power of destroying the vitality of bacteria. Grohman‡ next observed that not only the intravascular, but also the extravascular blood possessed this property. The first exact investigations of the subject were made by von Fodor.§ The systematic investigation of the bactericidal activity of blood-serum *in vitro* was next taken up by Flügge‖ and more particularly by Nuttall,** who found that different blood-serums possessed the power of killing bacteria in large numbers, but that the bactericidal power of the serum soon disappeared, after which the serum became a good culture medium for the very bacteria it had formerly destroyed. Metschnikoff objected to the observations, declaring that all the phenomena were ultimately

* "Proc. Royal Society of London," LXXXII, p. 357, 1904.

† "Jahresberichte der Schles. Ges. f. vaterl. Kultur," 1874

‡ "Untersuchungen aus dem physiol. Institut zu Dorpat," Dorpat, 1884; Krüger

§ "Centralbl. f. Bakt.," etc., 1890, VII, p. 753.

‖ "Zeitschrift für Hygiene," Bd. IV, S. 208.

** "Zeitschrift für Hygiene," Bd. IV, S. 353.

referable to the leukocytes, so Nuttall investigated pericardial fluid and the aqueous humor of the eye, which were also found to possess bactericidal powers.

The matter was next taken up by Buchner and his associates,* who showed that the blood-plasma and blood-serum possessed exactly the same bactericidal effects as the total blood. Buchner and Nuttall both showed that the exposure of the bactericidal fluids to a temperature of 56° C. for a few hours entirely destroyed their activity, though low temperatures were without effect upon them. Buchner found that the exposure of the serum to sunlight and oxygen also destroyed the bactericidal power. Neutralization of alkaline serum did not destroy its activity, but when the serum was dialyzed and the NaCl removed from it, the germicidal power was lost, to return again when it was restored. Buchner called the bactericidal principle *alexin.*

Many interesting facts were collected bearing upon the bactericidal substance or alexin. Thus Moro† showed that it was proportionally more active in sucking infants than in adults, and Ehrlich and Brieger‡ found that it passed from mother to offspring in the milk.

At first Buchner regarded alexin as an albumin, but later§ he came to look upon it as a proteolytic enzyme, this view no doubt resulting from an endeavor to explain the relation of alexin to immunity against intoxication, in which it was necessary to show that alexin not only killed bacteria, but also destroyed toxins.

Hankin‖ endeavored to show that there were differences between the substances destroying the bacteria and those acting upon their toxic products. To the whole group he applied the term *defensive proteids.* Those present in natural immunity he called *sozins,* those found in acquired immunity *phylaxins.* Sozins with bactericidal activity he further described as *mycosozins,* those with toxin-destroying activities as *toxosozins.* Phylaxins with bactericidal action were called *mycophylaxins;* those with toxin-destroying properties *toxophylaxins.*

* "Centralbl. f Bakt.," etc , 1889, Bd. v, S. 817; VI, S. 1; "Archiv für Hygiene," 1891, x, S. 727; "Centralbl. f. Bakt.," etc., 1890, VII, S. 76.

† "Jahresb. f. Kinderheilkunde," v, S. 396.

‡ "Zeitschrift für Hyg.," 1893, XIII, S. 336.

§ "Münch. med. Woch.," 1899

‖ "Centralbl. f. Bakt.," etc., XII, Nos. 22, 23: XIV, No. 25.

Metschnikoff found it unnecessary to change his ideas, and persisted in referring all the phenomena to the phagocytes or to enzymes derived from them.

At this point it will be evident to the reader that the phagocytic theory and the humoral theory contain indubitable evidence to show that they are important factors in defending the body against invading organisms, and that in each we see mechanisms operative in certain cases. But we have seen that both Metschnikoff and Buchner are obliged to strain a point in order to meet the requirements of increasing knowledge of the subject of immunity.

Thus, when we come to analyze Buchner's theory of alexins, we find that if natural immunity depends upon the ability of the alexins to destroy bacteria, that which takes place *in vitro* should correspond with that which takes place in the animal body, and that the invasion of the animal's body by bacteria should be accompanied by diminution of the bactericidal substance in its blood, which should be used up before the bacteria can be successful in their invasion. Experimental evidence is, however, at hand to show that these requirements may or may not be fulfilled.

Behring and Nissen* found that there was a definite relation between the bactericidal power of the blood *in vitro* and the resisting powers of a large number of animals studied, but Lubarsch† showed the remarkable exceptions of the rabbit, which is highly susceptible to anthrax, though its blood is highly bactericidal to the anthrax bacillus, and the dog, which is scarcely susceptible to anthrax, though its blood is scarcely bactericidal to the bacillus.

Flügge‡ found the bactericidal power of the blood greatly lessened in thirty-six hours after anthrax infection, and Nissen that a definite number of bacteria could be killed by a bactericidal serum, after which the alexin became inactive. The diminution of the bactericidal power was shown to occur both in the animal and in the test-tube. He also showed that the reactions of the bactericidal serums were specific, and that when a culture of one kind of bacteria was injected into an animal, the immediate effect was to diminish the activity of the serum for that species, though not necessarily for other species. The diminution of bac-

* "Zeitschrift für Hygiene," 1890, VIII, S. 412.

† "Centralbl. f. Bakt.," etc., 1889, VI, S. 481.

‡ "Zeitschrift für Hygiene," IV, S. 208.

tericidal energy was shown by him to depend upon the presence of the bacteria, as the injection of filtrates of bacterial cultures did not affect the bactericidal properties of the serum. This was a very important observation.

There is a correspondence between the behavior of the phagocytes and that of the body juices in animals with acquired immunity, in that the activity of the phagocytes toward the bacteria is increased, and the bactericidal activity of the serum intensified. The experiments of Pfeiffer, Pfeiffer and Kolle, and of Bordet upon the bacteriolysis taking place through factors brought out by experimental immunization have already been mentioned. But immunity is only partly explained by alexins, for in acquired immunity we commonly encounter an increased tolerance to bacteriotoxins and other toxins not in any way connected with bacteriolysis and evidently depending upon other defenses.

The tolerance to certain toxins is, of course, natural to many animals. There are also exceptional cases of natural immunity against toxins, however, that call our attention to certain new factors that become very important for consideration at this point. Thus Cobbett,* Roux and Martin,† and Bolton‡ have shown that horses, that cannot be supposed ever to have come into contact with diphtheria bacilli, vary considerably in their resistance to diphtheria toxin, and that the serum of the resisting horses contains something that destroys or neutralizes the toxin *in vitro*, as well as exerts a protective influence upon animals into which it is injected. This substance exerts no inimical action upon the diphtheria bacilli, beyond what a normal serum would do, therefore cannot be alexin, but must be antitoxin. Abel§ found that the blood of healthy men occasionally contained some substance capable of neutralizing diphtheria toxin; Stern found one normal serum capable of protecting against typhoid infection and Metschnikoff one that protected against cholera infection. Fischel and Wunschheim‖ found newly born babies immune against diphtheria, presumably because of the presence of a small quantity of demonstrable protective substance in the blood. These are, however, peculiar and exceptional cases.

* "Lancet," Aug. 5, 1899, II, p. 532.

† "Ann. de l'Inst. Pasteur," VIII, p. 615, 1894.

‡ "Jour. of Experimental Medicine," July, 1896, I, No. 5.

§ "Centralbl. f. Bakt.," etc., XVII, pp. 36, 1895.

‖ "Zeitschr. für Heilkunde," 1895, XVI, pp. 429–482.

The most suggestive and fascinating theory of immunity is that of Ehrlich, and is known as the "Seitenkettentheorie" or the "Lateral-Chain Theory."*

He began his studies by an investigation into the nature of toxins and their mode of action. The discovery that there was no constant relation between the intoxicating and antitoxin combining powers of diphtheria toxic bouillon led him to the conclusion that the toxin molecules possessed two different affinities, which he described as *haptophorous* or combining, and *toxophorous* or poisoning. The former were constant, the latter variable. The deterioration in the strength of the toxic filtrates of bouillon cultures of diphtheria bacilli was shown to depend upon the transformation of the toxin into toxoids which were not poisonous, and was shown to be quite independent of the antitoxin combining affinity of the filtrate which remained unaltered. The inevitable interpretation seemed to be the existence in the bouillon of the haptophorous and toxophorous groups described. Similar toxophorous and haptophorous groups were shown to exist in other toxins—tetanolysin, by Madsen, venoms, by Myers, and milk-curdling ferments by Morgenroth. The neutralizing action of the antibodies produced in the blood of animals immunized to these various substances depends upon the immediate and direct combination or union of haptophorous groups in the antibodies with corresponding haptophorous groups of the respective toxins or active bodies.

The physiological activities of toxins differ from those of alkaloids and other poisons differ in three fundamentals:

* The writings of Ehrlich and his associates are so numerous and scattered, and often so fragmentary, that instead of referring to the literature according to the method adopted in other parts of this work, the reader who desires to refer to the original articles can best do so by making use of the following: Ehrlich, "Die Werthbemessung des Diphtherie Heilserums," Klinisches Jahrbuch, 1897; Ehrlich, "Die Konstitution des Diphtheriegiftes," Deutsche med. Woch., 1898; "Gesammelte Arbeiten zur Immunitätsforschung," August Hirschwald, Berlin, 1904—this work contains the collected papers of Ehrlich and his associates; Aschoff, "Ehrlich's Seitenkettentheorie und ihre Anwendung auf die Künstlichen Immunusirungs-prozesse," Jena, 1902, and the chapter upon "Wirkung und Entstehung der Aktiven Stoffe im Serum noch der Seitenkettentheorie," by Ehrlich and Morgenroth in Kolle and Wassermann's "Handbuch der Pathogene Mikroorganismen," Jena, 1904, Gustav Fischer. Readers unacquainted with the German language may find the essential facts in Ehrlich's Croonian Lecture, Proceedings of the Royal Society of London, LXVI, 1900, p. 424, and in Welch's "Huxley Lecture," Medical News, LXXXI, 1902, 2, p. 721.

first in their ability to produce anti-bodies in the bodies of animals into which they are injected; second, the manifestation of poisonous action only after a definite incubation period, and, third, an extremely labile composition, by which the toxin becomes quickly transformed to toxoids.

A study of the physiological action of toxins upon the cells resulted in showing that certain definite specific affinities existed, and that the union of the toxin with the cell antedated the production of symptoms. In some cases it was even found possible to disconnect the anchored toxin by bringing to the cells haptophorous groups for which the haptophorous elements of the toxin molecule were known to have an active affinity. Dönitz determined the quantity of tetanus antitoxin which, injected into the circulating blood immediately after the toxin, absolutely neutralized

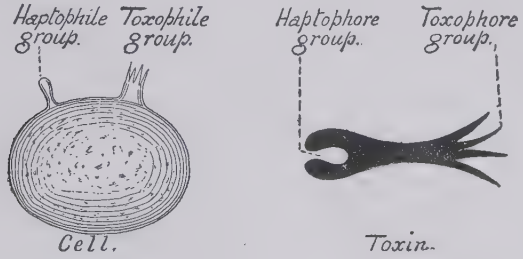


Fig. 7.—Diagram to represent the combining groups of the cell and of the toxin respectively (after Ehrlich) (Hewlett).

it and rendered all of the circulating toxin innocuous. If the same quantity of antitoxin was given seven or eight minutes after the injection of the toxin, death occurred from tetanus, exactly as if no antitoxin had been given. Evidently the toxin had anchored itself to the nerve-cells too quickly for the antitoxin to reach and combine with it. Heymans found that if an animal was injected with tetanus toxin, and its entire blood withdrawn immediately afterward and replaced by transfusion, it died of typical tetanus because in the brief interval between the toxin injection and the transfusion, the toxin molecules became anchored to the cell.

The ability of the cells thus to anchor the toxin is supposed by Ehrlich to depend upon the existence of haptophorous combining affinities, which he describes as *receptors*. He views the mode of toxin reception as depending upon a

mechanism either identical with or analogous to that by which cellular nutrition is maintained, and points out that in the case of methylene-blue and other colored substances, which afford an opportunity to make ocular observations upon the absorption of the pigment by the cells, only certain cells absorb the colors.

Cell nutrition is therefore probably carried on through the agency of receptors by which appropriate nutrient haptophorous groups are apprehended and utilized.

"We now come to the important question of the significance of the toxophile groups in organs. That these are in function especially designed to seize on toxins cannot be for one moment entertained. It would not be reasonable to suppose that there were present in the organism many hundreds of atomic groups, destined to unite with toxins, when the latter appeared, but in function really playing no part in the processes of normal life, and only arbitrarily brought into relation with them by the will of the investigator. It would, indeed, be highly superfluous, for example, for all our native animals to possess in their tissues atomic groups deliberately adapted to unite with abrin, ricin, and crotin, substances coming from far-distant tropics."

"One may, therefore, rightly assume that these toxophile protoplasmic groups in reality serve normal functions in the animal organism, and that they only incidentally and by pure chance possess the capacity to anchor themselves to this or that toxin."

"The first thought suggested by this assumption was that the atom group referred to must be concerned in tissue change; and it may be well here to sketch roughly the laws of cell metabolism. Here we must, in the first place, draw a clear line of distinction between those substances which are able to enter into the composition of the protoplasm, and so are really assimilated, and those which have no such capacity. To the first class belong a portion of the food-stuffs, *par excellence;* to the second almost all our pharmacological agents, alkaloids, antipyretics, antiseptics, etc."

"How is it possible to determine whether any given substance will be assimilated in the body or not? There can be no doubt that assimilation is in a special sense a synthetic process—that is to say, the molecule of the food-stuff concerned enters into combination with the protoplasm by a process of condensation involving loss of a portion of its

water. To take the example of sugar, in the union with protoplasm, not sugar itself as such, but a portion of it, comes into play, the sugar losing in the union some of its characteristic reactions. The sugar behaves here as it does, *e. g.*, in the glucosids, from which it can only be obtained through the agency of actual chemical cleavage. The glucosid shows no traces of sugar when extracted in indifferent solvents. In a quite analogous manner the sugar entering into the composition of albuminous bodies (glycoproteids) cannot be obtained by any method of extraction; at least not until chemical composition has previously taken place. It is, therefore, generally easy by means of extraction experiments, to decide whether any given combination in which the cells take part is, or is not, a synthetic one. If alkaloids, aromatic amines, antipyretics, or anilin dyes be introduced into the animal body, it is an easy matter, by means of water, alcohol, or acetone, according to the nature of the body, to remove all these substances quickly and easily from the tissues."

"This is most simply and convincingly demonstrated in the case of the anilin dyes. The nervous system stained with methylene-blue or the granules of the cells stained with neutral red at once yield up the die in the presence of alcohol. We are, therefore, obliged to conclude that none of the foreign bodies just mentioned enter synthetically into the cell complex; but are merely contained in the cells in their free state." . . . . "Hence with regard to the pharmacologically active bodies in general, it is not allowable to assume that they possess definite atom groups, which enter into combination with corresponding groups of the protoplasm. This corresponds, as I may remark beforehand, with the incapacity of all these substances to produce antitoxins in the animal body. We must, therefore, conclude that only certain substances, food-stuffs, *par excellence*, are endowed with properties admitting of their being, in the previously defined sense, chemically bound by the cells of the organism. We are obliged to adopt the view that the protoplasm is equipped with certain atomic groups, whose function especially consists in fixing to themselves certain food-stuffs of importance to the cell-life." We may assume that the protoplasm consists of a special executive center, in connection with which are nutritive side-chains, which possess a certain degree of independence and which

may differ from one another according to the requirements of the different cells. And as these side-chains have the office of attaching to themselves certain food-stuffs, we must also assume an atom-grouping in these food-stuffs themselves, every group uniting with a corresponding combining group of a side-chain. The relationship of the corresponding groups, *i. e.*, those of the food-stuff, and those of the cell, must be specific. They must be adapted to one another, as, *e. g.*, male and female screw (Pasteur), or as lock and key (E. Fischer). From this point of view, we must contemplate the relation of the toxin to the cell."

"We have already shown that the toxins possess for the antitoxins an attaching haptophore group, which accords entirely in its nature with the conditions we have ascribed to the relation existing between the food-stuffs and the cell side-chains. And the relation between toxin and cell ceases to be shrouded in mystery if we adopt the view that the haptophore groups of the toxins are molecular groups fitted to unite not only with the antitoxins, but also with the side-chains of the cells, and that it is by their agency that the toxin becomes anchored to the cells."

"We do not, however, require to suppose that the side-chains, which fit the haptophore group of the toxins, that is, the side-chains which are toxophile, represent something having no function in the normal cell economy. On the contrary, there is sufficient evidence that the toxophile side-chains are the same as those which have to do with the taking up of the food-stuffs by the protoplasm. The toxins are, in opposition to other poisons, of extremely complex structure, standing in their origin and chemical constitution in very close relationship to the proteids and their nearest derivatives. It is, therefore, not surprising that they possess a haptophore group corresponding with that of a food-stuff. Alongside of the binding haptophore group, which conditions their union to the protoplasm, the toxins are possessed of a second group, which in regard to the cell is not only useless, but actually injurious. And we remember that in the case of the diphtheria toxin there was reason to believe that there existed alongside of the haptophore group another and absolutely independent toxophore group." . . . . "As has been said, the possession of a toxophile group by the cell is the necessary preliminary and cause of the poisonous action of the toxin." . . . .

"If the cells of these organs [organs essential to life] lack side-chains fitted to unite with them, the toxophore group cannot become fixed to the cell, which therefore suffers no injury, *i. e.*, the organism is naturally immune. One of the most important forms of natural immunity is based upon the circumstance that in certain animals the organs essential to life are lacking in those haptophore groups which seize upon definite toxins. If, for example, the ptomaine occurring in sausages, which for man, monkeys, and rabbits is toxic in excessively minute doses, is for the dog harmless in quite large quantities, this is because the binding haptophore groups being wanting, the ptomaine cannot, in the dog, enter into direct relation with organs essential to life." . . . . "The haptophore group exercises its activity *immediately* after injection into the organism, while

Fig. 8.—Shows how the haptophores having united, the toxophores find a secondary adaptation to the cell, and so can poison it (after Ehrlich) (Hewlett).

in all toxins—with the perhaps solitary exception of snake-venom—the toxophore group comes into activity after the lapse of a longer or shorter incubation period which may, *e. g.*, in the case of diphtheria toxin, extend to several weeks."

"The theory above developed allows of an easy and natural explanation of the origin of antitoxins. In keeping with what has already been said, the first stage in the toxin action must be regarded as the union of the toxin by means of its haptophore group to certain 'side-chains' of the cell protoplasm. This union is, as animal experiments with a great number of toxins show, a firm and enduring one. The side-chain involved, so long as the union lasts, cannot exercise its normal nutritive physiological function—the taking up of food-stuffs. It is, as it were, shut out from participating, in the physiological sense, in the life of the cell. We

are, therefore, now concerned with a defect which, according to the principles so ably worked out by Professor Carl Weigert, is repaired by regeneration. These principles, in fact, constitute the leading conception of my theory. If after union has taken place, new quantities of toxin are administered at suitable intervals and in suitable quantities, the side-chains, which have been reproduced by the regenerative process, are taken up anew into union with the toxin, and so again the process of regeneration gives rise to the formation of fresh side-chains. In the course of the progress of typical systematic immunization, as this is practised in the case of diphtheria and tetanus toxin especially, the cells become, so to say, educated or trained to reproduce the necessary side-chains in ever-increasing

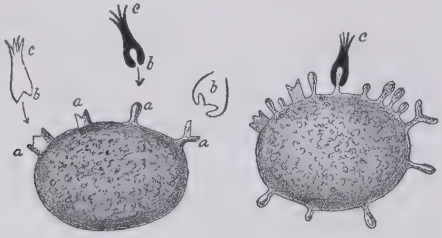
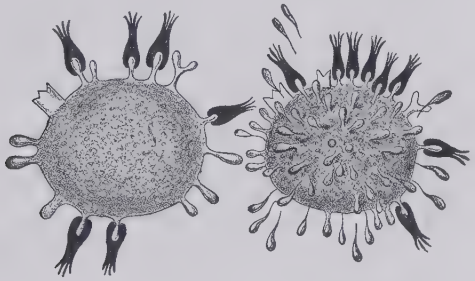


Fig. 9.—Cells with various receptors or haptophorous groups of the first order (*a*), adapted to combination with the haptophorous groups (*b*) of various chemical compounds brought to them. It will be noted that there is no mechanism by which the toxophorous elements of the molecules (*c*) can be brought to the cell.

quantity. As Weigert has confirmed by many examples, this, however, does not take place by the simple replacement of the defect; the compensation proceeds far beyond the necessary limit; indeed, overcompensation is the rule. Thus the lasting and ever-increasing regeneration must finally reach a stage at which such an excess of side-chains is produced that, to use a trivial expression, the side-chains are present in too great a quantity for the cell to carry and are, after the manner of a secretion, handed over as needless ballast to the blood. Regarded in accordance with this conception, the *antitoxins represent nothing more than side-chains reproduced in excess during regeneration and therefore pushed off from the protoplasm and so coming to exist in the free state.*"

The essence of Ehrlich's theory is tersely expressed by Behring: "The same substance which when incorporated in the cells of the living body, is the prerequisite and condition for an intoxication, becomes the means of cure when it exists in the circulating blood."



Figs. 10 and 11.—Show the regeneration of the cell-haptophores or receptors to compensate for the loss of those thrown out of service.

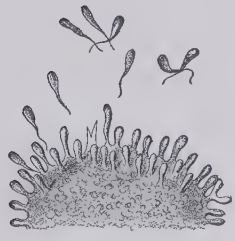


Fig. 12.—Shows the number of haptophores regenerated by the cell becoming excessive, they are thrown off into the tissue juice.

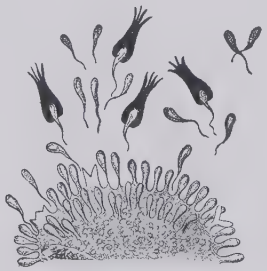


Fig. 13.—Explains what antitoxins are and how they are formed. The liberated receptors in the tissue juice and in the blood, possess identical combining affinities with those upon the cell, and meeting the adapted haptophorous elements in the blood, combine with them, thus keeping them from the cells.

Continuing the quotations from Ehrlich's Croonian Lecture, we find: "In the first place, our theory affords an explanation of the specific nature of the antitoxins, that tetanus antitoxin is only caused to be produced by tetanus toxin, and diphtheria antitoxin through diphtheria toxin.

This very specific nature of the affinity between toxin and cell is the necessary preliminary and cause of the toxicity itself. Further, our theory makes it easy to understand the long-lasting character of the immunity produced by one or several administrations of toxin, and also the fact that the organism reacts to relatively small quantities of toxin by the production of very much greater quantities of antitoxin." By the act of immunization, certain cells of the organism become converted into cells secreting antitoxin at the same rate as this is excreted. New quantities of antitoxin are constantly produced and so throughout a long period the antitoxin content of the serum remains nearly constant. The secretory nature of the formation of antitoxins has been very strikingly illustrated by the beautiful experiments of Salmonson and Madsen, who have shown that pilocarpine, which augments the secretion of most glands, also occasions in immunized animals a rapid increase in the antitoxin content of the serum."

"The production of antitoxins must, in keeping with our theory, be regarded as a function of the haptophore group of the toxin, and it is easy therefore to understand why, out of the great number of alkaloids, none are in a position to cause the production of antitoxins. Conversely, indeed, I recognize in this incapacity of the alkaloids, in opposition to the toxins, to produce antitoxins, a further and salient proof of the truth of the deduction I have previously based on chemical grounds, that the alkaloids possess no haptophore group which anchors them to the cells of organs. To formulate a general statement, the capacity of a body to cause the production of antitoxin stands in inseparable connection with the presence of a haptophore atomic group. In the formation of antitoxin the toxophore group of the toxin molecule is, on the contrary, of absolutely no moment. But the toxoid modification of the toxins, in which the haptophore group of the toxin is retained, while the toxophore group has ceased to be active, possesses the property of producing antitoxins. Indeed, in some cases of extremely susceptible animals, immunity can only be attained by means of the toxoids, and not by the too strongly acting toxins." . . . . "The symptoms of illness due to the action of the toxophore group, therefore, play no part in the production of antitoxin." The effect of enzymes upon the organism with the production of anti-bodies, and the "specific

precipitins" caused by the injection of milk, albumin, and peptones into animals may be looked upon as "having their origin in the most widely diverse organs, and representing nothing more than nutritive side-chains, which, in the course of the normal nutritive processes have been developed in excess and pushed off into the blood."

"Much more complex than in the cases hitherto discussed are the conditions when, instead of the relatively simple metabolic products of microbes, the living micro-organisms themselves come to be considered, as in immunization against cholera, typhoid, anthrax, swine-fever, and many other infectious diseases. Thus there come into existence alongside of the antitoxins produced as a result of the action of the toxins, manifold other reaction products. This is because the bacterium is a highly complicated living cell of which the solution in the organism yields a great number of bodies of different nature, in consequence of which a multitude of 'antikörper' are called into existence. Thus we see, as a result of the injection of bacterial cultures, that there arise alongside of the specific bacteriolysins, which dissolve the bacteria, other products, as, for example, the 'coagulins' (Kraus, Bordet), *i. e.*, substances which are able to cause the precipitation of certain albuminous bodies contained in the culture fluid injected; also the much-discussed agglutinins (Durham, Gruber, Pfeiffer), the antiferments (von Düngern), and no doubt many other bodies which have not yet been recognized. It is by no means unlikely that each of these reaction products finds its origin in special cells of the body; on the other hand, it is quite likely that the formation of any single one of these bodies is not of itself sufficient to confer immunity. Thus, in the case of the introduction of bacteria into the body we have to do with a many-sided production of different forms of 'antikörper,' each of which is directed only against one definite quality or metabolic product of the bacterial cell. Accordingly, in recent times, the practice of using for the production of immunization definite toxic bodies isolated from the bacterial cells has been more and more given up, and for this purpose it is now regarded as important to employ the bacterial cells as intact as possible." . . . . "The most interesting and important substances arising during such an immunizing process are without doubt the bacteriolysins." . . . . "Belfanti and Carbone first discovered the remarkable fact

that horses which had been treated with the blood-corpuscles of rabbits contain in their serum constituents which are poisonous for the rabbit, and for the rabbit only." . . . "Bordet showed shortly thereafter that in the case quoted there was present in the serum a specific hemolysin which dissolved the corpuscles of the rabbit. He also proved that these hemolysins—as had already been shown by Buchner and Daremberg in the case of similarly acting bodies which are present in normal blood—lost their solvent property on being maintained during half an hour at a temperature of 55° C. Bordet added, further, a new fact, that the blood-solvent property of those sera which had been deprived of solvent power by heat, the solvent action could be restored if certain normal sera were added to them. By this important observation an exact analogy was established with the facts of bacteriolysis as elicited by the work of Pfeiffer, Metschnikoff, and Bordet." . . . .

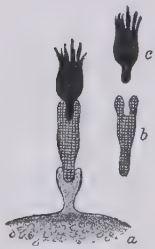


Fig. 14.—Combination of cell (*a*), amboceptor (*b*), and complement (*c*). The amboceptor may unite with the cell, but cannot affect it alone. The complement cannot unite with the cell except through the amboceptor, having no adaptation to the cell directly.

. . . "In collaboration with Dr. Morgenroth, I have sought in regard to this question, for which hemolysis offered prospects favorable to experimentation, to make clear the mechanism concerned in the action of these two compounds—the stable, which may be designated 'immune body,' and the unstable, which may be designated 'complement'—which acting together effect the solution of the red blood-corpuscles. For this purpose, in the first place, solutions containing either only the 'immune body' or only the 'complement' were brought in contact with suitable blood-corpuscles, and after separation of the fluid and the corpuscles by centrifugalization, we investigated whether these substances had been taken up by the red corpuscles or remained behind in the fluid. The proof of its location in the one position or in the other was readily forthcoming, since to restore the hemolysin to its former activity, it was only necessary to add to the 'immune body' a fresh supply of 'complement,' or to the 'complement' a fresh supply of 'immune body' in order that the presence of the hemolysin in its integrity

might be shown by the occurrence of solution of the red cells. The experiments proved that, after centrifugalizing, the 'immune body' is quantitatively bound to the red blood-corpuscles, and that the 'complement,' on the contrary, remains entirely behind in the fluid. The presence of the two components in contact with blood-corpuscles only occasions the solution of these at higher temperatures, and not at o° C. And an active hemolytic serum (with 'immune body' and 'complement' both present) having been placed in contact with red blood-corpuscles and maintained for a while at o° C., it was found after centrifugalizing that, under these circumstances also the 'immune body' had united with the red blood-corpuscles, but that the 'complement' remained in the serum. This experiment showed that both components must, at a temperature of o° C., have existed alongside of one another in a free condition." . . . .

"But when analogous experiments were undertaken at a higher temperature it was found that both components were retained in the sediment.

"These facts can only be explained by making certain assumptions regarding the constitution of the two components, *i. e.*, of the 'immune body' and the 'complement.' In the first place, two haptophore groups must be ascribed to the 'immune body,' one having affinity for a corresponding haptophore group of the red blood-corpuscles and with which at a lower temperature it quickly unites, and another haptophore group of a lesser chemical affinity, which at a higher temperature becomes united with the 'complement' present in the serum. Therefore at the higher temperature the red blood-corpuscles will draw to themselves those molecules of the 'immune body' which in the fluid have previously become united to the 'complement.' In this case the 'immune body' represents in a measure the connecting chain which binds the complement to the red blood-corpuscles and so brings them under its deleterious influence. Since under the influence of the 'complement'—at least, in the case of the bacteria—appearances are to be observed (for example, in the Pfeiffer phenomenon) which must be regarded as analogous to digestion, we shall not seriously err if we ascribe to this 'complement' a ferment-like character." . . . . "Having obtained a precise conception of the method of action of the lysins of the serum—of the hemolysins, and thereby also of the bacteriolysins—it be-

comes possible for us to attempt to solve the mystery of the origin of these bodies. I have in the beginning of this lecture* fully developed the 'side-chain theory,' according to which the antitoxins are merely certain of the protoplasm 'side-chain' which have been produced in excess and pushed off into the blood.

"The toxins as secretion products of the cells are in all likelihood still relatively uncomplicated bodies; at least by comparison with the primary and complex albumins of which the living cell is composed.

"If we now recognize that the different lysins arise only through absorption of highly complex cell material—such as red blood-corpuscles or bacteria—then the explanation, in accordance with what I have said, is that there are present in the organism 'side-chains' of a special nature, so constituted that they are endowed not only with an atomic group by virtue of the affinities of which they are enabled to pick up material, but also with a second atomic group, which, being ferment-loving in its nature, brings about the digestion of the material taken up. Should the pushing off of these 'side-chains' be forced, as it were, by immunization, then the 'side-chains' thus set free must possess both groups, and will, therefore, in their characteristics entirely correspond with what we have placed beyond doubt as regards the 'immune body' of the hemolysin."

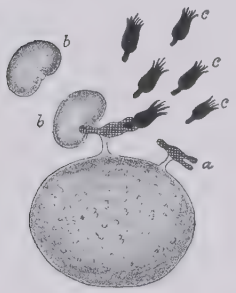


Fig. 15.—Cell with receptors of the second order (*a*) by which the cells fix useful molecules, of albumins, etc., on one hand (*b*), and zymogen molecules (*c*) on the other hand, and make use of the one substance through the action of the other.

An analysis of this theory shows complete natural immunity to depend upon the absence of haptophore groups (receptors) by which the toxins can be united to the cells. Extreme sensitivity or susceptibility probably depends upon the adapted haptophores being present or at least most numerous upon the cells of highly vital organs; com-

*These lengthy extracts, through which I have endeavored to enable Ehrlich to explain his theory to the reader, are taken from his "Croonian Lecture," delivered before the Royal Society of London, March 22, 1900.

parative insensitivity or insusceptibility upon the fact that the greater number of haptophore groups are attached to comparatively unimportant cells whose combining affinities have to be satisfied before combination with more vital cells can be accomplished. In some cases natural immunity is increased by the presence of free haptophore groups (antitoxin) in the blood.

Acquired immunity against toxins depends upon the regeneration of the cellular haptophores or receptors which, being liberated into the body juices, fix the haptophores of the toxin molecules before they are able to reach the cells themselves. Antitoxins and other anti-bodies, including the lysins, consist of liberated cellular haptophores or receptors, the former having a single combining affinity, the latter a double combining affinity, by which they unite, on the one hand, with the cell to be dissolved, on the other with the complement by which it is to be dissolved. Anti-bodies having this double combining affinity have been called "*amboceptors*" by Ehrlich. They are variously known in different writings as "immune bodies," amboceptors, *substance sensibilisatrice*, desmon, and *fixateur*. The "complement" or "addiment" of Ehrlich is also called alexin and cytase. Ehrlich conceives every amboceptor and every complement to be specific, but Bordet and others, while admitting that the amboceptor is specific, holds that there is but one complement or cytase.

It has already been shown that Metschnikoff's primitive conception of phagocytosis, that of the body being defended against the invasive power of the microparasites by the incorporation and digestion of the microparasites, had to be modified to conform to the increasing information upon the immunity reactions. He has persistently clung to the idea that the phagocytes are the essential factors. When invasive organisms enter the body, positive chemotactic influences determine that they are met by a sufficient number of phagocytes to devour and destroy them. If the invading organisms are too powerful and the phagocytes are killed by them and their toxins, the phagolysis or dissolution of the phagocytes liberates their enzymes into the blood. These liberated enzymes now act deleteriously upon the invaders, tending to agglutinate—aggregate them in clumps—and sensitize them to the future action of the phagocytes by which they may be taken up. Thus, the blood-serum ac-

quires a "fixing" or "sensitizing" quality from the presence of the "*fixateur*" or "*substance sensibilisatrice.*" The actual solution of the bacteria is accomplished by the "microcytase" of the leukocytic phagocytes. Thus, we find that Metschnikoff is prepared to account for the "amboceptor" or "immune body" of Ehrlich, which is the *fixateur*, and the "complement," which is the "*microcytase.*" When the phagolysis is excessive, either from infection or intoxication, both the fixateur and microcytase are free in the serum. In cases where the bacteria exert a negatively chemotactic influence upon the leukocytes, no immunity exists. The reactive phenomena occasioned by the introduction of tissue elements and blood-corpuscles, depend upon enzymes derived from the macrophages, the "*fixateur*" as before, and "*macrocytase.*"

The antitoxins are similarly accounted for: the cellular digestive enzymes exert their action not only upon the organized complex molecules of the microparasites, but also upon their more simple toxic products, fixing or otherwise altering them until they can be finally destroyed.

It will be seen that the two chief theories, though they appear discordant when explained independently of one another, are fairly well coördinated. Ehrlich believes the active bodies to be the products of those cells of the body for whose haptophorous combining groups the haptophorous groups of the active bodies engaged, and does not attribute the function to any particular group of cells; Metschnikoff attributes all the activities to the phagocytes, and especially the leukocytes. Ehrlich looks upon the phenomena as chemical and pictures them as taking places independently of the cells; Metschnikoff looks upon them as vital and brought about by the agency of living cells.

The fundamental ideas embodied in the "lateral-chain theory" of immunity may, by reversing the hypothesis and considering the bacterial instead of the body cells to be upon the defensive, be made to explain other phenomena of immunity. Walker* seems to have been the pioneer in this field, and his researches show that it is possible to immunize bacteria against "immune serums" by cultivating them in media containing increasing proportions of the immune serums. The bacteria thus cultivated were of increased virulence. The idea was further amplified by Welch in his Huxley Lecture.† The microorganismal cells must be regarded as endowed with receptors of their own, fitted for combination with adapted haptophorous elements in the juices reaching them, and therefore capable of reacting toward such

*"Jour. of Path. and Bact.," VIII, No. 1, p. 34, March, 1902.

†"British Medical Journal," Oct. 11, 1902, p. 1105; "Medical News," Oct. 18, 1902.

substances exactly as do the cells of the host. As the host reacts toward the active products of the bacteria, so the bacteria react toward the defensive products of the host, and as the cells of the former are stimulated to the production of immune bodies that shall facilitate bacteriolysis, so the latter are stimulated to antagonize their action by producing neutralizing bodies. These neutralizing bodies by which the defenses of the host are broken down are among those described by Bail* as "*aggressins*."

Thus, as the cells of the host invaded are constantly reacting to the active bodies produced by the invading parasites, so the latter are reacting toward the defensive products of the former. If the reactive processes of the host predominate, immunity and the destruction of the parasites result; if those of the bacteria predominate, increased virulence, facilitated invasion, and death of the host may result. This hypothesis also serves to make clear why micro-organisms entering the body not infrequently show a marked tendency to colonize in certain organs and tissues in preference to others.

Supposing accident to determine the tissue in which the primary infection has taken place, a longer or shorter residence in that tissue, with the resulting more or less marked acquired immunity against the defensive activities of that tissue, endow the organism with a higher degree of virulence for it than for other tissues, so that if at some future time the organism entering the circulation of a new host were able to colonize in any tissue of the body, its activities could be more easily and more successfully manifested in that to which it had already become accustomed, and to which it had acquired a peculiar adaptability. This adaptability has been made the subject of interesting experimental demonstration by Forssner† in his work upon the intravenous injection of streptococci.

SPECIAL PHENOMENA OF INFECTION AND IMMUNITY.

Certain phenomena which present themselves in the course of infection and immunity, to which reference has already been made, must now be considered in detail. These are *Specific Precipitation*, *Agglutination*, *Antibody Formation*, and *Cytolysis*.

I. Specific Precipitation.—In 1897 Kraus‡ in studying the "specific reactions produced by homologous serums with germ-free filtrates of bouillon cultures, of cholera, typhoid and plague bacteria," observed that immune serum brought into contact with the respective culture filtrate occasioned a precipitate specific in nature, to which he gave the name "specific precipitate." It thus became known that in addition to antitoxins, bacteriolysins, and agglutinins, other specific bodies were formed.

Bordet§ and Tchistowitch‖ showed that the phenome-

*"Wiener klin. Woch.," 1905, Nos. 9, 14, 16, 17; "Berl. klin. Woch.," 1905, No. 15; "Zeitschr. f. Hyg.," 1905, Bd. 1, No. 3.

†"Nordiskt Medicinskt Archiv," Bd. xxxv, p. 1, 1902.

‡"Wiener klin. Woch.," 1897, No. 32.

§"Ann. de l'Inst. Pasteur," 1899, p. 173.

‖"Ann. de l'Inst. Pasteur," 1899, p. 406.

non was of wide occurrence and had a broad significance, for they discovered that when the serum of one animal was injected into another animal some reaction took place by which the two serums being subsequently brought together precipitation took place. The same was found true of milk. Myers,* Jacoby,† Nolf,‡ and others showed that the faculty of provoking specific precipitins was common to many albuminous bodies—albumen, globulin, albumose, peptone, ricin, etc. Kraus in his original communication dwelt upon the specific nature of the precipitation, and was corroborated by Fish,§ Wassermann,‖ Morgenroth, and others, and it was shown that the reaction was sufficiently accurate to make possible the differentiation of human and goat's milk. The most important practical application, however, came through Uhlenhuth** and Wassermann,†† who made use of it for the differentiation of bloods for forensic purposes.

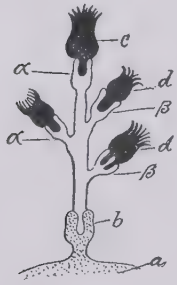


Fig. 16.—Polyceptor (Ehrlich and Marshall) such as can be conceived to occur in hemolysis and bacteriolysis where various complements are engaged. *a*, Receptor of bacterial cell; *b*, cytophil group of the amboceptor; *c*, dominating complement; *d*, subordinate complement; *α*, *β*, complementophil groups of the amboceptor, *α* for the dominating, *β* for the subordinate complements.

Uhlenhuth gave rabbits intraperitoneal injections of 10 c.c. of defibrinated blood at intervals of from six to eight days and found the blood-serum strongly precipitant after the fifth. He used such serum for testing the reaction with the bloods of oxen, horses, donkeys, pigs, sheep, dogs, cats, deer, hares, guinea-pigs, rats, mice, rabbits, chickens, geese, turkeys, pigeons, and men.

The method of making the test is important, as carelessness of detail will interfere with the accuracy of the result. The blood to be tested is diluted about 1 : 100, or until it has a feeble red color, with tap water, and then freed from

* "Centralbl. f. Bakt.," etc., 1900, Bd. xxxiii, and "The Lancet," 1900, ii, p. 98.

† "Archiv für exper. Path. u. Pharmak.," 1900.

‡ "Ann. de l'Inst. Pasteur," 1900, p. 297.

§ "Courier of Medicine," St. Louis, Feb., 1900.

‖ "Verhandl. d. Kong. f. innere Med.," 1900, S. 501, Wiesbaden.

** "Deutsche med. Woch.," 1900 and 1901.

†† "Samml. klin. Vortr. v. Volkman," Leipzig, Verlag von Breitkopf and Härtel, 1902.

corpuscular stroma by filtration or decantation. Two cubic centimeters of it are placed in a small test-tube, and further diluted with an equal quantity of physiological salt solution (if more water be added a precipitate of globulin might take place and spoil the experiment). To such a prepared blood preparation, from six to eight drops of the immune serum are added. If the diluted blood come from the same kind of an animal as that used to immunize the animal furnishing the test serum, immediate clouding takes place, and a flocculent precipitate forms. The precipitate never occurs with any other blood.

Wassermann and Schütze prepared a test serum by injecting rabbits with human blood, and tested its precipitating powers upon twenty-three other kinds of blood and found no reaction except with the blood of a baboon, but the reaction in that case was not nearly so marked as with human blood.

The most interesting and one of the most important biological applications of this phenomenon is by Nuttall, whose work, "Blood Immunity and Blood Relationship" (Cambridge, 1904), should be read by all who wish to study the subject for its scientific interest as a means of determining the blood relationship of animals, or its practical medico-legal importance in recognizing blood-stains. Nuttall comes to the following conclusions: (1) The investigations we have made confirm and extend the observations of others with regard to the formation of specific precipitins in the blood-serum of animals treated with various sera. (2) These precipitins are specific, although they may produce a slight reaction with the sera of allied animals. (3) The substance in serum which brings about the formation of a precipitin, as also the precipitin itself, are remarkably stable bodies. (4) The new test can be successfully applied to a blood which has been mixed with those of several other animals. (5) We have in this test the most delicate means hitherto discovered of detecting and testing bloods, and consequently we may hope that it will be put to forensic use."

The injection of the precipitinogenic serum into animals results in the formation of anti-precipitins by which their activity is neutralized.

II. Agglutination.—This phenomenon was first observed by Charrin and Roger* in the course of some experiments with Bacillus pyocyaneus. They found that the bacilli

* "Compte rendu de la Soc. de Biol.," 1899, p. 667.

when introduced into serum of animals infected with or immunized against cultures of Bacillus pyocyaneus ceased their active movements, became aggregated in clusters and settled to the bottom, leaving the fluid clear. Observations confirming and enlarging upon the observation were made by Metschnikoff,* Issaeff† and others. Gruber and Durham‡ made an elaborate and now classic study of the subject, first employing the term "agglutination" to the phenomenon, and "agglutinins" to the substances in the serum by which it might be brought about. They found that when cholera or typhoid bacilli are mixed with their respective immune serums, the organisms lose motility and become aggregated in clusters, masses or "clumps." They further showed the reaction to be specific within certain limitations, *i. e.*, typhoid immune serum agglutinated typhoid-like bacilli but no others, etc., and they saw in the phenomenon a practical means for the differentiation of different, closely related bacteria, an application that has, indeed, become a useful one.

It remained for Widal§ to show that it had a much more important application, in that the micro-organism being known, the effect produced by a serum upon it would be an indication of the past infection of the animal from which the serum was secured. The first practical application was made in connection with typhoid fever, and the brilliant success attending it had led to the test being known as the "Widal reaction" (*q. v.*).

The agglutinins are stable substances that resist drying and can be kept dry and active for years. Widal and Sicard found that they pass with difficulty through a porcelain filter and do not dialyze. They are precipitated in part by 15 per cent. of sodium chloride that throws down fibrinogen, and further precipitated with magnesium sulphate, which throws down globulins. They therefore think they are intimately related to the globulins and to fibrinogen. A temperature of 60° C. diminishes their activity, but they are not destroyed below 80° C. Sunlight has no effect upon them.

Metschnikoff looks upon agglutination as preliminary to phagocytosis and to bacteriolysis, and thinks it the effect of enzymes in the serum preparing and clustering the bacteria

* "Ann. de l'Inst. Pasteur," v, 1891.

† "Ann. de l'Inst. Pasteur," vii, 1893.

‡ "Münchener med. Woch.," 1896, No. 9.

§ "Société Médicale des Hôpitaux," June 26, 1896.

to be taken up by the phagocytes. Ehrlich finds in the agglutinins nothing more than receptors of what he denominates the II order, each of which possesses a zymophore and an agglutinophore group. (See Nothnagel's "Specielle Pathologie und Therapie," VIII, 1901.)

Malvoz* found that the addition of chemical substances, such as safranin, yesuvin, and corrosive sublimate, to cultures of the typhoid bacilli would cause their agglutination. Typhoid bacilli retained on the Chamberland filter and washed for a long time, could no longer be agglutinated, and were found to have lost their flagella and to be without motion, and led Dineur,† who made additional experiments, to conclude that agglutination depended upon the flagella. Malvoz‡ found that bacteria were sometimes agglutinated by their own metabolic products. He prepared a fresh culture of the first vaccine of the anthrax bacillus by thoroughly distributing it through ½ c.c. of distilled water, and then added a loopful of a six-day-old culture. After standing for a few hours typical agglutinations were observed under the microscope.

H. C. Ernst and Robey§ think that flagella have nothing to do with the flagella, which subsequent experiment has shown to be correct, as many non-flagellated bacteria can be agglutinated by their respective serums.

Boil,‖ Joos,** Eisenberg and Voll†† have shown that all of the agglutinins possess haptophore and agglutinophore groups, either of which may be destroyed without the other. Thus typhoid agglutinative serum when exposed to a temperature of 65° C. loses the agglutinophores, and no longer clumps the bacteria, though it retains the haptophores, and when brought into contact with the bacteria combines with them, producing no agglutination, but preventing the action of any other agglutinogenic serum.

Buxton and Vaughan‡‡ find that bacteria differ both in their agglutinogenic powers and their agglutinability, both of which must be taken into account in studying the subject.

Theobald Smith§§ has shown that there are two kinds of

* "Ann. de l'Inst. Pasteur," 1897, No. 6.
† "Bull. de l'Acad. de Méd. de Belgique," 1898, IV, p. 705.
‡ "Ann. de l'Inst. Pasteur," Aug. 25, 1899.
§ "Trans. Cong. Amer. Phys. and Surg.," 1900, p. 26.
‖ "Archiv f. Hyg.," 1902, XLII, Heft 4.
** "Zeit. f. Hyg.," XXXVI, 1901, p. 422.
†† "Zeitschr. f. Hyg.," XL, 1902, p. 155.
‡‡ "Jour. Med. Research," July, 1904.
§§ "Jour. Med. Research," 1904, vol. X, p. 89.

agglutinins, one of which acts upon the bacteria directly, the other through the flagella. The occurrence of these two bodies explains some of the incompatible results of previous experiments.

The reaction of agglutination has been applied with more or less success for the differentiation of all kinds of closely related bacteria, and in cases where the cultural and morphologic resemblances of closely related species are great, as in the case of the typhoid and para-typhoid bacilli (*q. v.*), has been found a valuable adjunct to the laboratory methods. It is also employed with great success in the diagnosis of typhoid and paratyphoid fever, various forms of dysentery, and with varying success in many other diseases.

The agglutinins when injected into animals effect definite chemico-physiological reactions with the formation of anti-bodies inhibiting their own activity.

III. The Antitoxins.—In the synopsis of immunity experiments already given the history of the discovery and development of the anti-bodies has been outlined, together with references to the original contributions in which they were made public. In the second part of this work the chapters dealing with the individual infectious diseases will contain additional information upon the mode of preparation, therapeutic action, etc., of the antitoxins in particular. At this point, however, it seems well to gather together a few general facts regarding the anti-bodies, without which many of their important practical bearings might be misunderstood.

In the section upon the "Explanation of Immunity" we have seen that the best mode of accounting for the occurrence of antitoxins is afforded by Ehrlich in the lateral-chain theory. He regards them as cell haptophores—receptors—that are found in excess of the requirements, by cells frequently stimulated by the presence of bacteria-products possessing adapted haptophores. The receptors are under normal conditions engaged in maintaining the proper nutrition of the cell; under abnormal conditions (as when preempted by the inert or injurious haptophores of the bacterio-products) are obliged to increase in number to compensate for the damage done the cell. The substances by which anti-body formation can be stimulated must bear a resemblance to the normal nutrient substances absorbed by the cells in that they are provided with haptophore groups corresponding with the haptophore groups of the cells and

so adapted for union with them. Mineral substances and alkaloidal substances have no such adaptations, but bacterial products, the toxalbumins of various higher plants, venoms, enzymes, and other proteid combinations have. The possession of the haptophore groups determines whether or not the cell can stimulate anti-body formation, and the ability to produce anti-bodies shows the existence of the haptophore groups.

The attachment of the haptophore groups to the cells is usually shown by morbid action of the cells in cases where there are associated toxophore groups, as in the case of the bacterio-toxins, but may not be discovered if there are no toxophore groups. The combination of the toxin-haptophores with the cell-haptophores can be demonstrated in the test-tube by crushing the cerebral substance of a rabbit, and adding tetanus toxin. The toxin becomes fixed by combination with the cell haptophores or receptors, loses its further combining powers and fails to affect animals into which it is subsequently injected. The increased formation of receptors in consequence of repeated stimulation has been shown by the effect of abrin upon the conjunctiva. If dropped into one eye until the conjunctiva is thoroughly immune against its action, the cells of this eye develop a greatly increased capacity for absorbing—*i. e.*, fixing—the abrin as compared with those of the other eye. Thus if the two conjunctival membranes be dissected out and a certain quantity of abrin triturated with each, the haptophores of the cells of the immunized membrane fix the poison so that it is no longer able deleteriously to affect animals, while no such effect takes place with the other membrane.

The ability to stimulate the formation of anti-bodies is entirely independent of any toxic action and is entirely the work of the haptophores. This is best shown in the fact that diphtheria toxin that has been heated or otherwise manipulated until its toxic action is lost, still retains the power of combining with antitoxin, or of producing anti-bodies.

The cells furnishing the haptophore groups or receptors whose presence in the blood gives it its antitoxic quality vary in number or quality in different animals. Thus, in the warm-blooded animals the rapidity with which tetanus toxin is anchored to the cells of the central nervous system seems to indicate that those cells, if not the only cells in the body passing the adapted receptors by which it is anchored,

are the chief cells by which it is absorbed. In the alligator, however, other cells seem to fix the toxin before it reaches or connects with those of the nervous system, so that the alligator is immune against the action of the toxin, though it is able to make antitoxin as well as susceptible animals.

Each introduction of appropriate anti-body forming substance is followed by an outpouring of the anti-body far in excess of what would neutralize it, so that after a systematic treatment has been carried out for some time, the neutralizing value of the blood may be *a thousand times* what would be necessary to neutralize the total quantity of active substance introduced into the animal.

Each anti-body is specific in action, as must be evident from its mode of formation. Should it be found, however, that several active bodies possessed haptophore groups of identical structure, the anti-body formed by any of them might be found to possess common neutralizing powers for all.

The animal whose blood contains anti-bodies enjoys immunity from the active body by which they were formed only so long as they are present. In some cases, however, animals that have been long subjected to the immunization treatment, and whose blood contains large quantities of free antitoxin, unexpectedly become abnormally sensitive (hypersensitivity) to the toxin, and may die after receiving a very small dose. This may be attributed to a difference in the combining activity of the receptors attached to the cells, and those separated and free in the serum. If the former developed a greater affinity for the toxin than the latter, it would unite with them by preference and intoxication ensue. If the treatment by which they are produced is interrupted, they immediately begin to lessen in quantity, and eventually disappear. Their occurrence in the blood determines that they shall be found in all the body juices.

Their chemical composition, which experiment shows to be of proteid nature, determines that when practical use is to be made of them, they must not be administered by the stomach, as digestion is usually followed by their destruction. In infants, the proteid digestion being feeble, antitoxins pass from the mother's milk to the sucking offspring without digestion, but the administration of antitoxins by this method at later periods of life is followed by effects too uncertain to be depended upon. For practical therapeutic purposes, therefore, the administration must always be made hypodermically or intravenously.

1. **Diphtheria Antitoxin.**—This was first utilized for practical therapeutic purposes by Behring.* An account of the method utilized for securing it in utilizable quantities will be found in the chapter upon diphtheria. As usually prepared by the administration of the toxin, it is essentially an antitoxin and has no destructive action upon the diphtheria bacilli. In therapeutics it is employed to neutralize or "fix" the toxin circulating in the blood, not to destroy the bacilli, or to effect the regeneration of the tissues injuriously acted upon by the toxin. Martin is of the opinion that such purely antitoxic serums are inferior to those containing other immunity products, such as bacteriolysins, and recommends that the whole culture instead of the filtered culture be used in the immunization of the animal. If this is done, the bacteriolytic effect is added to the antitoxic effects of the serum.

The serum may be used to prevent or to cure diphtheria (see chapter on Diphtheria).

2. **Tetanus antitoxin** was first prepared by Behring and Kitasato.† It can be employed for the prevention or cure of tetanus. For the former purpose, hypodermic injections of the serum may be given in cases with suspicious wounds, or the wounds may be dusted with a powder made by pulverizing the dried serum. For treatment the serum must be administered in frequently repeated large doses by hypodermic or intravenous injection. The results are less brilliant than those attained with diphtheria antitoxin because of the avidity with which the cells of the central nervous system take up the tetanus toxin, and the firmness of the union formed. An analysis of a great number of cases has, however, shown that the recoveries following the free administration of the serum exceed the recoveries effected by other methods of treatment by about 40 per cent. (see chapter upon Tetanus).

3. **Antivenene or Anti-venomous Serum.**—This was discovered by Phisalix and Bertrand‡ and made practical for therapeutic purposes by Calmette.§ Calmette found that cobra venom contained two principles, one of which, labile in nature and readily destroyed by heat, was destruc-

* "Die Blutserumtherapie," Berlin, 1902.

† "Deutsche med. Wochenschrift," 1890, No. 49.

‡ "Compt. rendu de l'Acad. des Sciences de Paris," Feb. 5, 1894, Tome CXVIII, p. 356.

§ "Compt. rendu de la Soc. de Biol. de Paris," 10 Series, Tome I, p. 120, Feb. 10, 1904.

tive in action upon the tissues with which it came into direct contact, the other stable in nature, was death-dealing through its action upon the respiratory centers. By heating the venoms and thus destroying the irritative principle, he was able to immunize animals against the other, which he looked upon as the important element of the venom. The immunized animals furnished an anti-serum, which entirely annulled the effect of the toxin (modified venom) used in treating them. This serum was found to protect rabbits and other animals against both modified and unmodified cobra venom, and was used successfully in the treatment of a number of human beings that had been bitten by cobras. Calmette, however, drew the erroneous conclusion that because in most venoms studied, he was able to find a larger or smaller proportion of the respiratory poison, it constituted the essential element of the venom, and that to be antagonized. Arguing from this standpoint, he recommended his antivenene in all cases of snake-bite, regardless of the variety of serpent. C. J. Martin and others showed that Calmette was wrong in his deductions, and that his antivenene was of very doubtful benefit in the treatment of the bites of the Australian serpents, and my own experiments showed it to be of doubtful value in the treatment of the bites of the American snakes. In the venoms of our snakes—the rattlesnake, copper-head, and moccasin—the poison is essentially locally destructive in action, the fatal influence upon the respiratory centers being of secondary importance, fatalities from snake-bite being rare in the United States. Although I made many attempts to immunize horses against this locally destructive substance, which has since been carefully studied by Flexner and Noguchi, I was unable to do so. Noguchi, however, applied Ehrlich's principle to the investigation, destroyed the toxophorous group of the venom molecules, and succeeded in producing an anti-serum useful in antagonizing the active principle—*hemorrhagin*—of the Crotalus venom.

Antivenene is useful in the treatment of cobra invenomation, as Calmette has shown by cases treated in his own laboratory. The serums of Noguchi and others are equally useful in their respective invenomations, but the opportunity for successfully employing antivenenes is very small. Few persons are bitten in places and at times where the remedy is at hand, and the effects of venoms of all kinds are so rapid that immediate treatment is required. In India and a few

other reptile infected countries, as well as in zoological gardens where venomous serpents are kept, and in laboratories where the snakes are kept for experimental purposes, it is well to be provided with a supply of the serum, but it has no wide sphere of usefulness.

4. **Miscellaneous anti-bodies** of many kinds have been experimentally produced,—anti-enzymes, etc.,—but have no practical application. A knowledge of them is, however, essential to a thorough understanding of the reactions of immunity. In the synopsis of the experiments upon immunity reference is made to the bodies, and to the literature bearing upon them.

IV. The Cytotoxins.—These comprehend the bacteriolysins, hemolysins, spermotoxins, epitheliolysins, nephrotoxins, etc. They appear after immunization against formed elements, according to principles made clear in the section explaining immunity. The reactions involved in their operation result from the combination of the "amboceptor," "immune body," "*substance sensibilisatrice*," "*fixateur*," or "desmon" with the "addiment," "complement," "alexin," or "cytase." It was at first hoped that bacteriolytic serums would have a wide therapeutic application in all cases in which non-toxicogenic bacteria were invading the body, but experiment and experience have shown that the laws governing their action greatly limit their application, and that their effects, when not beneficial, are bound to be harmful.

It has already been explained that when bacteria of any kind are repeatedly injected into an animal, its serum soon comes to contain a considerable number of haptophore groups fitted to combine with micro-organisms of similar kind and so make possible the action of the real destroying substance, the complement. In this process, however, the complement itself is not increased, so that the serum has in itself scarcely any increase of bactericidal activity above the normal, though it may serve to activate other serums not normally active upon that bacterium. To introduce an immune body into the blood of an animal suffering from an infection of any kind is to do it little good, because in most cases the existing infection has brought about the formation of as much or more "immune body" than can be utilized by the complement, and more cannot be utilized. To give it injections of active bodies that cannot be utilized is shown by

Comus and Gley* and Kossel† to be followed by the formation of anti-bodies—in this case "anti-immune bodies"—by which their effect is neutralized. Should anti-immune bodies be found by this meddlesome medication, the state of the infected animal would be worse than before, because it would now be preparing that which by neutralizing the combining affinities of its own immune bodies, would prevent them from combining with the elements to be destroyed and so activating the complements.

No satisfactory method of experimentally increasing the complement has been devised. If, as Metschnikoff supposes, the complement is microcytase derived from disintegrated leukocytes, aseptic suppurations with active phagolysis should result in marked increase of the complement. As a

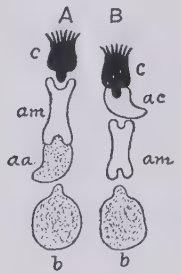


Fig. 17.—Schematic representation of the interfering action of anamboceptors and anti-complements. A, Anti-amboceptor action: *c*, Complement; *am*, amboceptor; *aa*, antiamboceptor preventing the amboceptor from connecting with the cell. B: *c*, Complement; *ac*, anticomplement preventing the complement from connecting with the amboceptor, *am*.

matter of fact, this does take place, but the increase is so slight that the serum is not practically valuable.

Therapeutic serums whose practical application is based upon their cytolytic activity must, of necessity, contain both the essential factors involved in cytolysis, and should contain them in such proportions that, regardless of other elements in the blood, they can exercise their combining and dissolving functions.

We are unable experimentally to accomplish these prerequisites, therefore are not in the position to accurately apply bacteriolytic serums in practice.

* "Compte rendu de l'Acad. de Sciences de Paris," Jan. 1, 1898, 126.
† "Berl. klin. Woch.," 1898, S. 152.

CHAPTER V.

METHODS OF OBSERVING BACTERIA.

It is of the utmost importance to examine bacteria alive and as nearly in their normal environment as possible, then to supplement this examination by the study of dead and stained specimens.

The study of the living organism has the advantage of showing its true shape, size, grouping, motility, reproduction, and natural history. It has the disadvantage of being somewhat difficult because of its small size and transparency.

So long as bacteria were observed only in the natural condition, however, it was impossible to find them in the tissues of diseased animals, and it was not until Weigert suggested the use of the anilin dyes for coloring them that their demonstration was made easy and their relationship to pathologic conditions established.

The beauty and clearness of stained specimens, and the ease with which they can be observed, have led to some serious errors on the part of students, who often fail to realize the unnatural condition of the stained bacteria they observe. It only needs a moment's consideration to show how disturbed must be the structure of an organism after it has been dried, fixed, boiled, or steamed, passed through several chemic reagents, dehydrated and impregnated with stains, etc., to suggest how totally unnatural its appearance may become.

It is therefore necessary to examine every bacterium, under study, in the living condition, and to control all the appearances of the stained specimen by comparison.

I. THE STUDY OF LIVING BACTERIA.

The simplest method of observing live bacteria is to take a drop of liquid containing them, place it upon a slide, put on a cover, and examine.

While this method is simple, it cannot be recommended, as evaporation at the edges causes currents of liquid to flow to and fro beneath the cover, carrying the bacteria with them and making it almost impossible to determine whether the organisms under examination are motile or not. Should it be desirable that such a specimen be kept for a time, so much evaporation takes place that in the course of an hour or two it has changed too much to be of further use.

The best way to examine living micro-organisms is in what is called the *hanging drop* (Fig. 18). A hollow-ground slide is used, and with the aid of a small camel's-hair pencil a ring of vaselin is drawn on the slide about, not in, the concavity at its center. A drop of the material to be examined is placed in the center of a large clean cover-glass

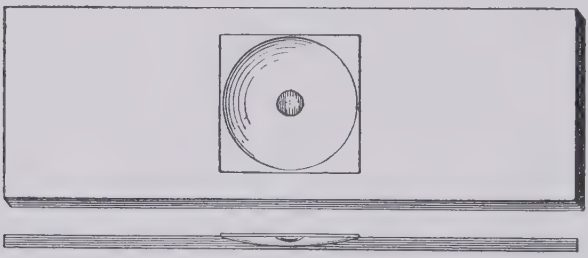


Fig. 18.—The "hanging drop" seen from above and in profile.

and then placed upon the slide so that the drop hangs in, but does not touch, the concavity. The micro-organisms are thus hermetically sealed in an air chamber, and appear under almost the same conditions as in the culture. Such a specimen may be kept and examined from day to day, the bacteria continuing to live until the oxygen or nutriment is exhausted. By means of a special apparatus (Fig. 19), in which the microscope is placed, the growing bacteria may be watched at any temperature, and very exact observations made.

The hanging drop should always be examined at the edge, as the center is too thick.

In such a specimen it is possible to determine the shape, size, grouping, division, sporulation, and motility of the organism under observation.

Care should be exercised to use a rather small drop, especially for the detection of motility, as a large one vibrates and masks the motility of the sluggish forms.

When the bacteria to be observed are in solid or semi-solid culture, a small quantity of the culture should be mixed in a drop of sterile bouillon or other fluid.

For observing the growth of bacteria where it is desirable to prevent movement, Hill * has invented an ingenious device which he calls the "hanging block." His directions for preparing it are as follows:

"Pour melted nutrient agar into a Petri dish to the depth of about one-eighth or one-quarter inch. Cool this agar, and cut from it a block about one-quarter inch to one-third inch square and of the thickness of the agar layer in the dish. This block has a smooth upper and under surface. Place it, under side down, on a slide and protect it from dust. Prepare an emulsion, in sterile water, of the organism to be examined if it has been grown on a solid medium, or use a broth culture; spread the emulsion or broth upon the upper surface of the block as if making an ordinary cover-slip preparation. Place the slide and block in a 37° C. incubator for five to ten minutes to dry slightly. Then lay a clean sterile cover-slip on the inoculated surface of the block in close contact with it, usually avoiding air-bubbles. Remove the slide from the lower surface of the block and invert the cover-slip so that the agar block is uppermost. With a platinum loop run a drop or two of melted agar along each side of the agar block, to fill the angles between the sides of the block and the cover-slip. This seal hardens at once, preventing slipping of the block. Place the preparation in the incubator again for five or ten minutes to dry the agar-agar seal. Invert this preparation over a moist chamber and seal the cover-slip in place with white wax or paraffin. Vaselin softens too readily at 37° C., allowing shifting of the cover-slip. The preparation may then be examined at leisure."

With this means of examining the growing cultures, Hill has acquired interesting knowledge of the fission and budding of Bacillus diphtheriæ.

If the specimens to be examined must be kept for some time at an elevated temperature, some such apparatus as that of Nuttall will be found useful though expensive.

II. THE STUDY OF STAINED BACTERIA.

In the early days of bacteriology efforts were made to facilitate the observation of bacteria by the use of nuclear dyes. Both carmin and hematoxylin tinge the nuclei of the bacteria a little, but so unsatisfactorily that since Weigert introduced the anilin dyes for the purpose, all other stains

* "Journal of Medical Research," vol. VII, No. 2; new series, vol. II, March, 1902.

have been abandoned. The affinity between the bacteria and the anilin dyes is peculiar, and in certain cases can be used for the differentiation of species.

The best anilin dyes made at the present time, and those which have become the standard for all bacteriologic work, are made in Germany by Dr. Grübler, and in ordering stains the name of this manufacturer should be specified.

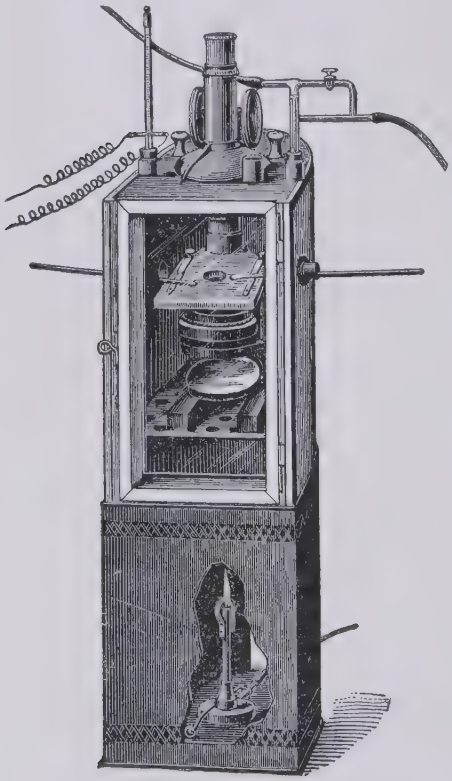


Fig. 19.—Apparatus for keeping objects under microscopic examination at constant temperatures (Nuttall).

A whole volume could easily be devoted to the technic of staining. Indeed, the difficulties encountered are so great that no explanations can be too thorough to be useful. Readers interested in the biochemistry of the subject will do well to refer to the excellent papers by Arnold Grimme,* upon "The Important Methods of Staining Bacteria, etc.," and Marx,† upon "The Metachromatic and Babes-Ernst Granules."

In this work special methods for staining such bacteria as have peculiar reactions will be given together with the description of the particular organisms, general methods only being discussed in this chapter.

* "Centralbl. f. Bakt.," etc., Bd. XXXII, Nos. 2, 3, 4, and 5, 1902.
† *Ibid.*, XXXII, Nos. 10 and 11, p. 108, 1902.

Preparations for General Examination.—For bacteriologic purposes thin covers (No. 1) are required, because thicker glasses may interfere with the focussing of the oil-immersion lenses. The cover-glasses must be *perfectly clean.* It is therefore best to clean a large quantity in advance of use by immersing them first in a strong mineral acid, then washing them in water, then in alcohol, then in ether, and finally keeping them in ether until they are to be used. Except that it sometimes cracks, bends, or fuses the edge of the glass, a more convenient method is to wipe the glasses as clean as possible with a soft cotton cloth, seize them with *fine-pointed* forceps, and pass them repeatedly through a small Bunsen flame until it becomes greenish-yellow. The hot glass must then be slowly elevated above the flame, so as to allow it to anneal. This manœuver removes the organic matter by combustion. It is not expedient to use covers twice for bacteriologic work, though if well cleansed by immersion in acid and washing, they may subsequently be employed for ordinary microscopic objects.

The fragility of the covers and their likelihood to be broken or dropped at the critical moment, make most workers prefer to stain directly upon the slide. The slide should be thoroughly cleaned, and if the material to be examined is spread near one end, the other may serve as a convenient handle. The slide is also to be preferred if a number of examinations are to be made simultaneously or for comparison, as it is large enough to contain a number of "smears."

Simple Method of Staining.—The material to be examined must be spread in the thinnest possible layer upon the surface of the perfectly clean cover-glass or slide and dried. The most convenient method of spreading is to place a minute drop on the glass with a platinum loop, and then spread it evenly over the glass with the flat wire. Should it be stained at once it would all wash off, so it must next be fixed to the glass by being passed *three times through a flame*, experience having shown that when drawn through the flame three times the desired effect is usually accomplished. The Germans recommend that a Bunsen burner or a large alcohol lamp be used, that the arm describe a circle a foot in diameter, each revolution occupying a second of time, and the glass being made to pass through the flame

from apex to base three times. This is supposed to be exactly the requisite amount of heating. The rule is a good one for the inexperienced.

Inequality in the size of various flames may make it desirable to have a more accurate rule. Novy * suggests that so soon as it is found that the glass is so hot that it can no longer be held against the finger it is sufficiently heated for fixing.

After fixing, the preparation is ready for the stain. Every laboratory should be provided with *stock solutions* of the ordinary dyes. These are *saturated alcoholic* solutions made by adding 25 grams of the dye to 100 c.c. of alcohol. Of these it is well to have fuchsin, gentian violet, and methylene-blue always made up. The stock solutions will not stain, but form the basis of the staining solutions. For ordinary staining an *aqueous solution* is employed. A small bottle is nearly filled with distilled water, and the stock

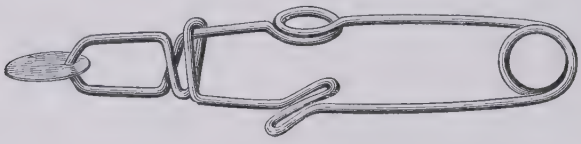


Fig. 20.—Stewart's cover-glass forceps

solution added, drop by drop, until the color becomes just sufficiently intense to prevent the ready recognition of objects through it. For exact work it is probably best to give these stains a standard composition, using 5 c.c of the saturated alcoholic solution to 95 c.c. of water. Such a watery solution possesses the power of readily penetrating the dried cytoplasm of the bacterium.

Cover-glasses are apt to slip from the fingers and spill the stain, so when using them it is well to be provided with special forceps (Fig. 20), which hold the glass in a firm grip and allow of all manipulations without danger of soiling the fingers or clothes. The ordinary sharp-pointed forceps are unfit for the purpose, as capillary attraction draws the stain between the blades and makes certain the soiling of the fingers. In using the special forceps the glass should not be caught at the edge, but a short distance from it, as shown in the cut. This altogether prevents capillary at-

* "Laboratory Work in Bacteriology" 1899

traction between the blades. Sufficient stain is allowed to run from a pipet upon the smear to flood it, but not overflow, and is allowed to remain for a moment or two, after which it is thoroughly washed off with water. If the specimen is prepared for temporary use, it can be dried and examined at once, a drop of oil of cedar being placed directly upon the smear, and no cover-glass used. If the staining has been done upon a cover-glass it can be mounted upon a slide with a drop of water between, and then examined, though this is less satisfactory than examination after mounting in Canada balsam.

Sometimes the material to be examined is solid or too thick to spread upon the glass conveniently. Under such circumstances a drop of distilled water or bouillon can be added and a minute portion of the material mixed in it and spread upon the glass.

When the bacteria are contained in urine or other non-albuminous fluid, so that the heat used for fixing has nothing to coagulate and fix the organisms to the glass, a drop of Meyer's glycerin-albumen can be added with advantage, though the precaution must be taken to see that this mixture contains no bacteria to cause confusion with those in the material to be studied.

The entire process is, in brief: (1) Spread the material upon the glass; (2) dry—do not heat; (3) pass three times through the flame; (4) stain—one minute; (5) wash thoroughly in water; (6) dry; (7) mount in Canada balsam.

To Observe Bacteria in Sections of Tissue.—Hardening.—It not infrequently happens that the bacteria to be examined are scattered among or inclosed in the cells of tissues. Their demonstration then becomes a matter of difficulty, and the method employed must be modified according to the particular kind of organism. The success of the method will depend upon the good preservation of the tissue to be studied. As bacteria disintegrate rapidly in dead tissue, the specimen for examination should be secured as fresh as possible, cut into small fragments, and immersed in absolute alcohol from six to twenty-four hours, to kill and fix the cells and bacteria. The blocks are then removed from the absolute alcohol and kept in 80 to 90 per cent. alcohol, which does not shrink the tissue. Solutions

of bichlorid of mercury* may also be used and are particularly useful when the bacteria are to be studied in relation to the cells of the tissues.

Tissues preserved in 95 per cent. alcohol, Müller's fluid, 4 per cent. formaldehyd, and other ordinary solutions rarely show the bacteria well.

Embedding.—The ordinary *methods of embedding* suffice. The simpler of these are probably as follows:

I. Celloidin (Schering).—The solutions of celloidin are made in equal parts of absolute alcohol and ether and should have the thickness of oil or molasses. From the hardening reagent (if other than absolute alcohol) pass the blocks of tissue through:

Ninety-five per cent. alcohol, twelve to twenty-four hours;
Absolute alcohol, six to twelve hours;
Thin celloidin (consistence of oil), twelve to twenty-four hours;
Thick celloidin (consistence of molasses), six to twelve hours.

Place upon a block of vulcanite or hard paraffin, allow the ether to evaporate until the block can be overturned without dislodging the specimen; then place in 80 per cent. alcohol until ready to cut. The knife must be kept flooded with alcohol while cutting.

Celloidin is soluble in absolute alcohol, ether, and oil of cloves, so that the staining of the sections must be accomplished without the use of these reagents if possible.

Celloidin sections can be fastened to the slide, if desired, by firmly pressing filter paper upon them and rubbing hard, then allowing a little vapor of ether to run upon them.

II. Paraffin.—Pure paraffin having a melting-point of about 55° C. is used. The hardened blocks of tissue are passed through:

* Zenker's fluid:

Bichromate of potassium	2.5 grams
Sulphate of sodium	1.0 gram
Bichlorid of mercury	5.0 grams
Water	100.0 "

At the time of using add 5 grams of glacial acetic acid. Permit the specimens to remain in the solution for a few hours only, then wash for twenty-four hours in running water and transfer to 80 per cent. alcohol.

Ninety-five per cent. alcohol, twelve to twenty-four hours;
Absolute alcohol, six to twelve hours;
Chloroform, benzole, or xylol, four hours;
A saturated solution of paraffin in one of the above reagents, four to eight hours.

The block is then placed in melted paraffin in an oven or paraffin water-bath, at 50°–60° C., until the volatile reagent is all evaporated, and the tissue impregnated with paraffin (four to twelve hours), and finally embedded in freshly melted paraffin in any convenient mold. In cutting, the knife must be perfectly dry.

The cut paraffin sections can be placed upon the surface of slightly warmed water to flatten out the wrinkles, and then floated upon a clean slide upon which a film of Meyer's glycerin-albumen (equal parts of glycerin and white of egg thoroughly beaten up and filtered, and preserved with a crystal of thymol) has been spread. After drying, the slides are placed in the paraffin oven for an hour at 60° C., so that the albumen coagulates and fixes the sections to the glass.

When sections so spread and fixed upon the slide are to be stained, the paraffin must first be dissolved in chloroform, benzole, xylol, oil of turpentine, etc., which in turn must be removed with 95 per cent. alcohol. The further staining, by whatever method desired, is accomplished by dropping the reagents upon the slide.

III. Glycerin-gelatin.—As the penetration of the tissue by celloidin is attended with deterioration in the staining qualities of the tubercle bacillus, it has been recommended by Kolle * that the tissue be saturated with a mixture of glycerin, 1 part; gelatin, 2 parts; and water, 3 parts; cemented to a cork or block of wood, hardened in absolute alcohol, and cut as usual for celloidin with a knife wet with alcohol.

Staining.—Simple Method.—For ordinary work the following simple method can be recommended: After the sections are cut and cemented to the slide, the paraffin and celloidin should be removed by appropriate solvents. The sections are immersed in the ordinary aqueous solution of the anilin stain and allowed to remain about five minutes, next washed in water for several minutes, then decolorized

* Flügge's "Die Mikroorganismen," vol. 1, page 534.

in 0.5 to 1 per cent. acetic acid solution. The acid removes the stain from the tissues, but ultimately from the bacteria as well, so that one must watch carefully, and so soon as the color has almost disappeared from the sections, they must be removed and transferred to absolute alcohol. At this point the process may be interrupted to allow the tissue elements to be countercolored with alum-carmin or any stain not requiring acid for differentiation, after which the sections are dehydrated in absolute alcohol, cleared in xylol, and mounted in Canada balsam.

The greater number of applications can be made by simply dropping the reagents upon the slide while held in the fingers. Where exposure to the reagents is to be prolonged, the Coplin jar (Fig. 21) or some more capacious device must be employed.

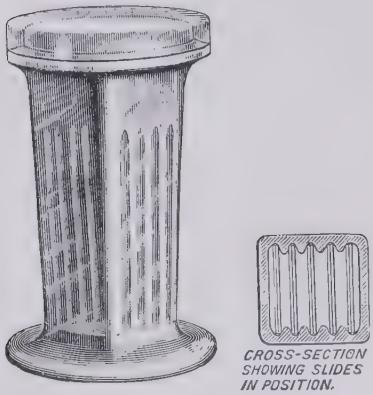


Fig 21 —Coplin's staining jar.

Pfeiffer's Method.—The sections are stained for one-half hour in diluted Ziehl's carbol-fuchsin (pp. 150 and 311), then transferred to absolute alcohol made feebly acid with acetic acid. The sections must be carefully watched, and so soon as the original, almost black-red color gives place to a red-violet color they are removed to xylol, to be cleared preparatory to mounting in balsam.

Löffler's Method.—Certain bacteria that do not permit ready penetration by the dye require some more intense stain. One of the best of these is Löffler's alkaline methylene-blue:

Saturated alcoholic solution of methylene-blue.. 30
1 : 10,000 aqueous solution of caustic potash ..100

The cut sections of tissue are stained for a few minutes and then differentiated in a 1 per cent. solution of hydrochloric acid for a few seconds, after which they are dehydrated in alcohol, cleared in xylol, and mounted in balsam.

Bacteria, such as the typhoid fever bacillus, which decolorize rapidly, do not require the use of acid for the differentiation, washing in water or alcohol being sufficient.

Gram's Method of Staining Bacteria in Tissue.—Gram was the fortunate discoverer of a method of impregnating bacteria with an insoluble color. It will be seen at a glance that this is a marked improvement on the methods given above, as the stained tissue can be washed thoroughly in either water or alcohol until its cells are colorless, without fear that the bacteria will be decolorized. The details of the method are as follows: The section is stained from five to ten minutes in a solution of a basic anilin dye, pure anilin (anilin oil) and water. This solution, first devised by Ehrlich, is known as Ehrlich's solution. The ordinary method of preparing it is to mix the following:

Pure anilin	4
Saturated alcoholic solution of gentian violet	11
Water	100

Instead of gentian violet, methyl violet, Victoria blue, or any pararosanilin dye will answer. The rosanilin dyes, such as fuchsin, methylene-blue, vesuvin, etc., will not react with iodin, and so cannot be used for the purpose. The anilin-oil solutions do not keep well; in fact, seldom longer than six to eight weeks, sometimes not more than two or three; therefore it is best to prepare but a small quantity by pouring about 1 c.c. of pure anilin into a test-tube, filling the tube about one-half with distilled water, shaking well, then filtering as much as is desired into a small dish. To this the saturated alcoholic solution of the dye is added until the surface becomes distinctly metallic in appearance.

Friedländer recommends that the section remain from fifteen to thirty minutes in warm stain, and in many cases the prolonged process gives better results.

From the stain the section is given a rather hasty washing in water, and then immersed from two to three minutes in Gram's solution (a dilute Lugol's solution):

Iodin crystals	1
Potassium iodid	2
Water	300

The specimen while in the Gram solution turns a dark blackish-brown color, but when removed and carefully washed in 95 per cent. alcohol again becomes blue. The washing in 95 per cent. alcohol is continued until no more color is given off and the tissue assumes its original color. If it is simply desired to find the bacteria, the section can be dehydrated in absolute alcohol for a moment, cleared in xylol, and mounted in Canada balsam. If it is necessary to study the relation of the bacteria to the tissue elements, a nuclear stain, such as alum-carmin or Bismarck brown, may be previously or subsequently used. Should a nuclear stain requiring acid for its differentiation be desirable, the process of staining must precede the Gram stain, so that the acid shall not act upon the stained bacteria.

Gram's method rests upon the fact that *the combination of mycoprotein, anilin dye, and the iodids forms a compound insoluble in alcohol.*

The process described may be summed up as follows:

Stain in Ehrlich's anilin-water gentian violet five to thirty minutes;
Wash in water;
Immerse two to three minutes in Gram's solution;
Wash in 95 per cent. alcohol until no more color comes out;
Dehydrate in absolute alcohol;
Clear in xylol;
Mount in Canada balsam.

This method stains the majority of bacteria, but not all, hence can be used to aid in the differentiation of similar species. Some bacteria that do not stain are important:

Spirillum choleræ asiaticæ;
Spirillum choleræ gallinarum;
Bacillus mallei (of glanders);
Bacillus œdematis maligni;
Bacillus pneumoniæ (Friedländer);
Micrococcus gonorrhœæ (Neisser);
Spirochæte obermeieri (of relapsing fever);
Bacillus typhosus;
Bacillus dysenteriæ;
Bacillus suipestifer;
Bacillus coli communis;
Bacillus icteroides;
Bacillus of rabbit septicemia;

Bacillus anthracis symptomatici;
Bacillus influenzæ;
Bacillus pestis bubonica;
Bacillus rhinoscleromatis;
Spirillum tyrogenum;
Spirillum choleræ nostras;
Spirillum metschnikovi.

No matter how carefully the method is performed, an unsightly precipitate is sometimes deposited upon the tissue, obscuring both its cells and contained bacteria. Muir and Ritchie obviate this (1) by making the staining solution with 1 : 20 aqueous solution of carbolic acid instead of the saturated anilin solution, and (2) by clearing the tissue with oil of cloves after dehydration with alcohol. The oil of cloves, however, is itself a powerful decolorant and must be washed out in xylol before the section is mounted in Canada balsam.

Gram's method is also employed to aid in differentiating similar species of bacteria in culture. A thin layer of a suspension of the bacteria to be examined is spread upon a slide or cover-glass, dried, and fixed; then flooded with the anilin-oil, gentian violet or other staining solution. The solution is kept warm by holding the glass flooded with the stain over a small flame. The process of staining is continued from two to five minutes. If the heating causes the stain to evaporate, more of it must be added so that it does not dry and incrust the glass.

The stain is poured off, and replaced by Gram's solution, which is allowed to remain from one-half to two minutes, and gently agitated.

The smear is next washed in 95 per cent. alcohol until the blue color is wholly or almost lost, after which it can be counterstained with eosin, Bismarck brown, vesuvin, etc., washed, dried, and mounted in Canada balsam. Given briefly, the method is:

Stain with Ehrlich's solution two to five minutes;
Gram's solution for one-half to two minutes;
Wash in 95 per cent. alcohol until decolorized;
Counterstain if desired; wash off the counterstain with water;
Dry;
Mount in Canada balsam.

The Gram-Weigert Stain can be employed with beautiful

results for staining many micro-organisms. It differs from the Gram method in that anilin oil instead of alcohol is used for decolorizing. To secure the most brilliant results it is best first to stain the tissue with alum, borax, or lithium carmin, and then—

1. Stain in Ehrlich's anilin-oil-water gentian violet, five to twenty minutes;
2. Wash off excess with normal salt solution;
3. Immerse in dilute iodin solution (iodin 1, iodid of potassium 2, water 100) for one minute;
4. Drain off the fluid and blot the section spread out upon the slide, with absorbent paper;
5. Decolorize with a mixture of equal parts of anilin and xylol;
6. Wash out the anilin with pure xylol;
7. Mount in xylol balsam.

Eosin and Methylene-blue (Mallory) make a beautiful contrast tissue stain for routine work, and also demonstrates the presence of most bacteria. The success of the method seems to depend largely upon the quality of the reagents used and a careful study of their effects. Hardening in Zenker's fluid is highly recommended as a preliminary. The details as given by Mallory are as follows:

1. Stain paraffin sections in a 5 to 10 per cent. aqueous solution of eosin for five to twenty minutes or longer;
2. Wash in water to get rid of the excess of eosin;
3. Stain in Unna's alkaline methylene-blue solution (methylene-blue 1, carbonate of potassium 1, water 100), diluted 1 : 10 with water, for one-half to one hour, or use a stronger solution and stain for a few minutes only;
4. Wash in water.
5. Differentiate and dehydrate in 95 per cent. alcohol, followed by absolute alcohol until the pink color returns in the section;
6. Clear with xylol;
7. Mount in xylol balsam.

The nuclei and micro-organisms will be colored blue, the cytoplasm, etc., red.

Zieler* recommends for the staining of the typhoid, glanders and other difficultly stainable bacteria, the following method of demonstration in the tissues:-

1. Fix and harden in Müller-formol solution.
   Paraffin imbedding.
2. Staining overnight in Orcein D. (Grübler), ... 0.1
   Officinal "schwefelsaüre" (sulphuric acid), ... 2.0
   70 per cent. alcohol, ... 100.0
3. Washing in 70 per cent. alcohol for a short time to remove the excess of orcein.
4. Washing in water.
5. Staining in polychrome methylene-blue ten minutes to two hours.
6. Washing in distilled water.
7. Thorough differentiation in glycerin-ether 1:2–5 water until the tissues become pale blue.
8. Washing in distilled water.
9. Seventy per cent. alcohol.
10. Absolute alcohol.
11. Xylol.
12. Balsam.

Glanders bacilli appear dark violet on a colorless background; typhoid bacilli intense dark red violet.

Method of Staining Spores.—It has already been pointed out that the peculiar quality of the spore capsules protects them to a certain extent from the influence of stains and disinfectants. On this account they are much more difficult to color than the adult bacteria. Several methods are recommended, the one generally employed being as follows: Spread the thinnest possible layer of material upon a cover-glass, dry, and fix. Have ready a watch-crystalful of Ehrlich's solution, preferably made of fuchsin, and drop the cover-glass, prepared side down, upon the surface, where it should float. Heat the stain until it begins to steam, and allow the specimen to remain in the hot stain for from five to fifteen minutes. The cover is then transferred to a 3 per cent. solution of hydrochloric acid in absolute alcohol for about one minute. Abbott recommends that the cover-glass be submerged, prepared side up, in a dish of this solution and gently agitated for exactly one minute, removed, washed in water, and counterstained with an aqueous solution of methyl or methylene-blue.

In such a specimen the spores should appear red, and the adult organisms blue.

* "Centralbl. f. allg. Path. u. path. Anat.," Bd. XIV, No. 14, p. 561.

I have not found that spores usually color so easily, and for many species the best method seems to be to place the prepared cover-glass in a test-tube half full of carbol-fuchsin:

Fuchsin	1
Alcohol	10
Five per cent. aqueous solution of phenol crystals	100

and boil it for at least fifteen minutes, after which it is decolorized, either with 3 per cent. hydrochloric or 2–5 per cent. acetic acid, washed in water, and counterstained blue.

Muir and Ritchie * recommend that cover-films be prepared and stained as for tubercle bacilli (*q. v.*), decolorized with a 1 per cent. sulphuric acid solution in water or methyl alcohol, then washed in water and counterstained with a saturated aqueous methylene-blue solution for half a minute, washed again with water, dried, and mounted in Canada balsam.

Möller † finds it advantageous to prepare the films, before staining, by immersion in chloroform for two minutes, following this by immersion in 5 per cent. chromic acid solution for one-half to two minutes.

Anjeszky ‡ recommends the following method of staining spores, which is said always to give good results even with anthrax bacilli: A cover-glass is thinly spread with the spore-containing fluid and dried. While it is drying, some 0.5 per cent. hydrochloric acid is warmed in a porcelain dish over a Bunsen flame until it steams well and bubbles begin to form. When the solution is hot and the smear dry, the cover-glass is dropped upon the fluid, which is allowed to act upon the unfixed smear for three or four minutes. The cover is removed, washed with water, dried, and fixed for the first time, then stained with Ziehl's carbol-fuchsin solution, which is warmed twice until fumes arise. The preparation is allowed to cool, decolorized with a 4–5 per cent. sulphuric acid solution, and counterstained for a minute or two with malachite green or methylene-blue. The whole procedure should not take longer than eight to ten minutes.

* "Manual of Bacteriology," London, 1897.

† "Centralbl. f. Bakt. u. Parasitenk.," Bd. x, p. 273.

‡ *Ibid.*, Feb. 27, 1898, XXIII, No. 8, p. 329.

Fiocca* suggests the following rapid method: "About 20 c.c. of a 10 per cent. aqueous solution of ammonium are poured into a watch-glass, and 10 to 20 drops of a saturated solution of gentian violet, fuchsin, methyl blue, or safranin added. The solution is warmed until vapor begins to rise, then is ready for use. A very thinly spread cover-glass, carefully dried and fixed, is immersed for three to five minutes (sometimes ten to twenty minutes), washed in water, washed momentarily in a 20 per cent. solution of nitric or sulphuric acid, washed again in water, then counter-stained with an aqueous solution of vesuvin, chrysoidin, methyl blue, malachite green, or safranin, according to the color of the preceding stain. This whole process is said to take only from eight to ten minutes, and to give remarkably clear and beautiful pictures."

Method of Staining Flagella.—This is somewhat more difficult than the staining of the bacteria or their spores.

Löffler's Method.†—This is the original and best method, though somewhat cumbersome, and hence rarely employed at the present time. Three solutions are used:

(A)—

Twenty per cent. aqueous solution of tannic acid	10
Cold saturated aqueous solution of ferrous sulphate	5
Alcoholic solution of fuchsin or methyl violet	1

(B) One per cent. aqueous solution of caustic soda.

(C) An aqueous solution of sulphuric acid of such strength that 1 c.c. will exactly neutralize an equal quantity of solution B.

Some of the culture to be stained is mixed upon a cover-glass with a drop of distilled water making a first dilution, which is still too rich in bacteria to permit the flagella to show well, so that it is recommended to prepare a second by placing a small drop of distilled water, upon a cover and taking a loopful from the first dilution to make the second, and spreading it over the entire surface without much rubbing or stirring. The film is allowed to dry, and is then fixed by passing it three times through the flame. When this is done with forceps there is some danger of the preparation becoming too hot, so Löffler recommends that

* "Centralbl. f Bakt. u. Parasitenk.," July 1, 1893, XIV, No. 1.
† *Ibid.*, 1890, Bd. VII, p. 625.

the glass be held in the fingers while the passes through the flame are made.

The cover-glass is now held in forceps, and the mordant, solution A, dropped upon it until it is well covered, when it is warmed until it begins to steam. The mordant must be replaced as it evaporates. It must not be heated too strongly; above all things, must not boil. This solution is allowed to act from one-half to one minute, is then washed off with distilled water, and then with absolute alcohol until all traces of the solution have been removed. The real stain,—Löffler recommends an anilin-water fuchsin (Ehrlich's solution),—which should have a neutral reaction, is next dropped on so as to cover the film, and heated for a minute until vapor begins to arise, after which it is washed off carefully, dried, and mounted in Canada balsam. To obtain the neutral reaction of the stain, enough of the 1 per cent. sodium hydrate solution is added to an amount of the anilin-water-fuchsin solution having a thickness of several centimeters to begin to change the transparent into an opaque solution.

A specimen thus treated may or may not show the flagella. If not, before proceeding further it is necessary to study the chemic products of the micro-organism in culture media. If by its growth the organism elaborates alkalies, from 1 drop to 1 c.c. of solution C in 16 c.c. must be added to the mordant A, and the staining repeated. It may be necessary to stain again and again until the proper amount is determined by the successful demonstration of the flagella. On the other hand, if the organism by its growth produces acid, solution B must be added, drop by drop, and numerous stained specimens examined to see with what addition of alkali the flagella will appear. Löffler fortunately worked out the amounts required for some species, and of the more important ones the following solutions of B and C must be added to 16 c.c. of solution A to attain the desired effect:

Cholera spirillum .....½–1 drop of solution C
Typhoid fever .....1 c.c. of solution B
Bacillus subtilis.....28–30 drops of solution B
Bacillus of malignant edema .36 or 37 drops of solution B

Part of the success of the staining depends upon having the bacteria thinly spread upon the glass, and as free from

albuminous and gelatinous materials as possible. The cover-glass must be cleaned most painstakingly; too much heating in fixing must be avoided. After using and washing off the mordant, the preparation should be dried before the application of the anilin-water-fuchsin solution.

Pitfield's Method.—Pitfield * has devised a simple and excellent method of staining flagella, a single solution at once mordant and stain being employed. It is made in two parts, which are filtered and mixed:

(A)—

Saturated aqueous solution of alum	10 c.c.
Saturated alcoholic solution of gentian violet	1 "

(B)—

Tannic acid	1 gr.
Distilled water	10 c.c.

The solutions should be made with cold water, and immediately after mixing the stain is ready for use. The cover-slip is carefully cleaned, the grease being burned off in a flame. After it has cooled, the bacteria are spread upon it, well diluted with water. After drying thoroughly in the air, the stain is gradually poured on and by gentle heating brought almost to a boil; the slip covered with the hot stain is laid aside for a minute, then washed in water and mounted.

Van Ermengem's Method.—Van Ermengem † has devised a somewhat complicated method of staining flagella, which has given great satisfaction. Three solutions, which he describes as the *bain fixateur*, *bain sensibilisateur*, and *bain reducteur et reinforçateur*, are to be used as follows:

1. *Bain fixateur:*

2 per cent. solution of osmic acid	1 part
10–25 per cent. solution of tannin	2 parts

The cover-glasses, which are very thinly spread, dried, and fixed, are placed in this bath for one hour at the room temperature, warmed until steam arises, and then kept hot for five minutes. They are next washed with distilled

* "Med. News," Sept. 7, 1895.

† "Travaux du Lab. d'hygiene et des bact. de Gand.," t. I, p. 3. Abstracted in the "Centralbl. f. Bakt. u. Parasitenk.," 1894, Bd. xv, p. 969.

water, then with absolute alcohol, then again with distilled water. All three washings must be very thorough.

2. *Bain sensibilisateur:*

5 per cent. solution of nitrate of silver in distilled water.

The films are allowed to remain in this for a few seconds, and are then immediately transferred to the third bath.

3. *Bain reducteur et reinforçateur:*

Gallic acid	5 grams
Tannin	3 "
Fused potassium acetate	10 "
Distilled water	350 c.c.

The preparations are kept in this solution for a few seconds, then returned to the nitrate of silver solution until they begin to turn black. They are then washed, dried, and mounted.

Mervyn Gorden modifies the method by allowing the preparations to remain in the second bath for two minutes, transferring to the third bath for one and a half to two minutes, and then washing, drying, and mounting without returning to the second bath.

Muir and Ritchie find it advantageous to use a fresh supply of the third solution for each specimen.

Measurement of Bacteria.—Bacteria can best be measured by an eyepiece micrometer. As these instruments vary somewhat in construction, the unit of measurement for each objective magnification and the method of manipulating the instruments must be learned from dealers' catalogues.

Photographing Bacteria.—Photographing bacteria requires special apparatus and methods. For a sufficient description of the necessary technic it is necessary to refer to special text-books upon the subject.*

* See the excellent chapter upon Photomicrography in Aschoff and Gaylord's "Pathological Histology." Philadelphia, 1900.

CHAPTER VI.

STERILIZATION AND DISINFECTION.

BEFORE considering the cultivation of bacteria and the preparation of media for that purpose, it is necessary to have a thorough knowledge of the principles of sterilization and disinfection in order intelligently to comprehend the methods employed for the destruction of those bacteria whose accidental presence might ruin our experiments.

The dust of the atmosphere, as has already been shown, is almost invariable in its micro-organismal contamination, so that spores of micro-organisms constantly settle from it upon our glassware, pots, kettles, funnels, etc., and would certainly ruin every medium with which we experiment did we not take appropriate measures for their destruction.

To get rid of these undesirable "weeds" we make use of our knowledge of the conditions destructive to bacterial life and subject the articles contaminated by them to the action of heat beyond their known enduring power, or to the action of chemic agents known to destroy them, or remove them from fluids into which they have entered by passage through unglazed porcelain.

Micro-organisms may be killed by heat or by the action of chemicals, the process being generically termed *sterilization*. The term sterilization is, however, technically employed to denote the destruction of micro-organisms by heat, in contradistinction to disinfection, which means the destruction of the micro-organisms by the use of chemic agents. A chemic agent causing the death of micro-organisms is called a *germicide*. Certain substances whose action is detrimental to the vitality of micro-organisms and prevents their growth amid otherwise suitable surroundings are termed *antiseptics*. An object which is entirely free from micro-organisms and their spores is said to be *sterile*.

The following list of germicides is chiefly extracted from the work of Sternberg and Micquel:

Pyoktanin (methyl violet)...1 : 2,000,000—1 : 5000.
Formalin (40 per cent. solution of formaldehyd) .....1 : 25,000—1 : 5000.
Hydrogen peroxid .....1 : 20,000.
Bichlorid of mercury .....1 : 14,300.
Nitrate of silver .....1 : 12,500.
Creolin .....1 : 5000—1 : 200.
Chromic acid .....1 : 5000.
Thymol .....1 : 1340.
Salicylic acid .....1 : 1000.
Potassium bichromate .....1 : 909.
Trikresol .....1 : 1000—1 : 500.
Zinc chlorid .....1 : 526.
Potassium permanganate ...1 : 285.
Nitrate of lead .....1 : 277.
Izal .....1 : 200.
Boracic acid.....1 : 143.
Chloral hydrate .....1 : 107.
Ferrous sulphate .....1 : 200—1 : 90.
Calcium chlorid .....1 : 25.
Creosote .....1 : 20.
Carbolic acid .....1 : 20 :: 1 : 50.
Chloretone .....saturated solution.
Alcohol .....1 : 10.
Ether. Pure ether will not kill anthrax spores immersed in it for eight days.

In a more recent study of disinfectants, Burgess * gives the following comparisons:

Biniodid of mercury .....1 : 5000.
Bichlorid of mercury.....1 : 2000.
Chlorinated lime.....1 : 100.
Formaldehyd.....1 : 40.
Lysol .....1 : 20.
Carbolic acid.....1 : 20.

As biniodid of mercury forms no combination with albumin, Burgess regards it as the best general disinfectant.

The value of antiseptics, like that of disinfectants, is always relative, the destructive as well as the inhibitory power of the solution varying with the micro-organism upon which it acts. The following table, from Boer, will illustrate this:

METHYL VIOLET (PYOKTANIN).

	Restrains.	Kills.
Anthrax bacillus	1 : 70,000	1 : 5000
Diphtheria	1 : 10,000	1 : 2000
Glanders	1 : 2500	1 : 150
Typhoid	1 : 2500	1 : 150
Cholera spirillum	1 : 30,000	1 : 1000

* "Lancet," June 23, 1900.

The study of sterilization, disinfection, and antisepsis may be divided as follows:

I. The sterilization and protection of instruments and glassware.

II. The sterilization and protection of culture media.

III. The disinfection of the instruments, ligatures, etc., and the hands of the surgeon, and the use of antiseptics.

IV. The disinfection of sick-chambers and their contents, as well as the dejecta and discharges of patients suffering from contagious and infectious diseases.

I. The Sterilization and Protection of Instruments and Glassware.—Sterilization may be accomplished by either moist or dry heat. For the perfect sterilization of objects capable of withstanding it dry heat is always to be preferred, because of its more certain action. If we knew just what organisms we had to deal with, we might be able in many cases to save time and gas; but though some non-spore-producing forms are killed at a temperature of 60° C., spore-bearers may withstand 100° C. for an hour; it is therefore best to employ a temperature high enough to kill all with certainty. The sterilizing apparatus, or "hot-air sterilizer," shown in figure 22, is made with three walls. The gas jets are inclosed within the space between the outer and middle walls, *C*, and can be seen at *F*. The heat ascends, warming the air between the two inner walls, which ascends between the walls, *K*, then descends over the contents, *J*, and escapes through perforations in the bottom, *B*, to supply the draught at *F*, and eventually escape again at *S*. *R* is the gas regulator, *T* the thermometer.

Platinum wires used for inoculation are sterilized by being held in the direct flame until they become incandescent. In sterilizing the wires attention must be bestowed upon the glass handle, which should be flamed for at least half its length for a few moments when used for the first time each day. Carelessness in this respect may result in the contamination of the cultures.

Knives, scissors, and *forceps* may be exposed for a very brief time to the direct flame, but as this affects the temper of the steel when continued too long, they are better boiled, steamed, or carbolized.

All articles of *glassware* are to be sterilized by an exposure of one-half to one hour to a sufficiently high temperature—

150° C. or 302° F.—in an appropriate hot-air oven. This temperature is fatal to all forms of microscopic life.

Rubber stoppers, corks, wooden apparatus, and other objects which are warped, cracked, charred, or melted by so high a temperature must be sterilized by exposure to streaming steam or steam under pressure, in the steam sterilizer or autoclave, before they can be pronounced sterile.

It must always be borne in mind that after sterilization

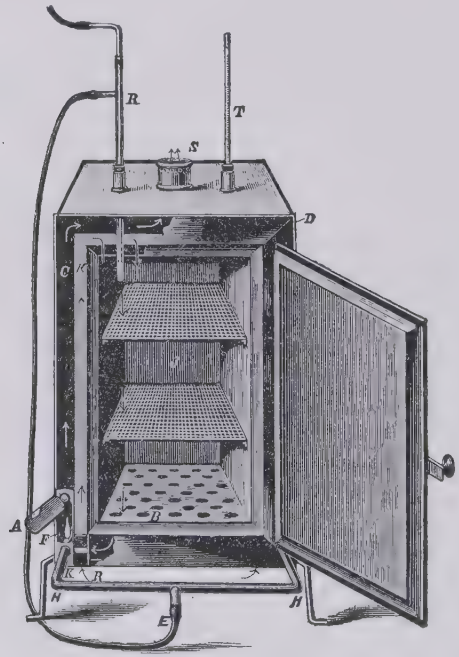


Fig. 22.—Hot-air sterilizer.

has been accomplished the original sources of contamination are still present, so that it is necessary to protect the sterilized objects and media from them.

To Schröder and Van Dusch belongs the credit of having first shown that when the mouths of flasks and tubes are closed with plugs of sterile cotton no germs can filter through. This discovery has been of inestimable value, and has been one of the chief means permitting the advance of bacteri-

ology. Before sterilizing, flasks and tubes are therefore carefully plugged with ordinary (non-absorbent) cotton-wool; they will then remain free from the access of germs until opened. Instruments may be sterilized wrapped in cotton, to be opened only when ready for use; or instruments and rubber goods sterilized by steam can subsequently be wrapped in sterile cotton and kept for use. It is of the utmost importance to carefully protect every sterilized object, in order that the object of the sterilization be not defeated. As the spores of molds falling upon cotton some-

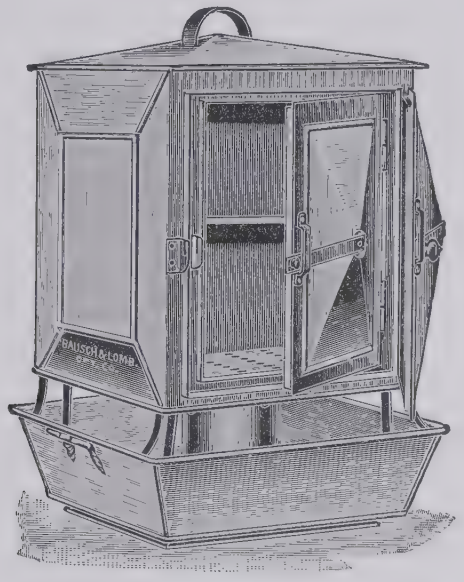


Fig. 23.—Arnold's steam sterilizer (Boston Board of Health form).

times grow and allow their mycelia to work their way through and drop into the culture-medium. Roux has employed paper caps (Fig. 24) with which the cotton stoppers can be protected from the dust. These are easily made by curling a small square of paper into a "cornucopia," and fastening by turning up the edge or putting in a pin. The paper is placed over the stopper before the sterilization, after which no contamination of the cotton can occur.

II. Sterilization and Protection of Culture Media.—As almost all of the culture media contain about 80 per

cent. of water, which would evaporate in the hot-air closet, and so destroy the material, hot-air sterilization is inappropriate for them, sterilization by streaming steam being the only satisfactory method. The prepared media are placed in previously sterilized flasks or tubes, carefully plugged with cotton-wool, and then sterilized in what is known as Koch's steam apparatus (Fig. 25) or in Arnold's steam sterilizer (Fig. 23), which is more convenient and more generally useful.

The temperature of boiling water, 100° C., does not kill the spores, so that one exposure of the culture media to streaming steam is of little use. The sterilization must be applied in a systematic manner—*intermittent sterilization*—based upon a knowledge of sporulation.

Fig. 24.—Flask with the cotton stopper protected with a paper cap.

Fig. 25.—Koch's steam sterilizer.

In carrying out intermittent sterilization the culture medium is exposed for fifteen minutes to the passage of streaming steam or to some temperature judged to be sufficiently high, so that the adult micro-organisms contained in it are killed. As the spores remain uninjured, the medium is stood aside in a cool place for twenty-four hours, and the spores allowed slowly to develop into adult organisms.

When the twenty-four hours have passed, the medium is again exposed to the same temperature, until these newly

developed bacteria are also killed. Eventually, the process is repeated a third time, lest a few spores remain alive. When properly sterilized in this way, culture media will remain free from contamination indefinitely.

In popular parlance, the intermittent exposure of the culture media to steam is spoken of as sterilization; a prolonged single exposure to lower temperatures (60°–70° C.), as *Pasteurization.*

Pasteurization is employed for the destruction of bacteria

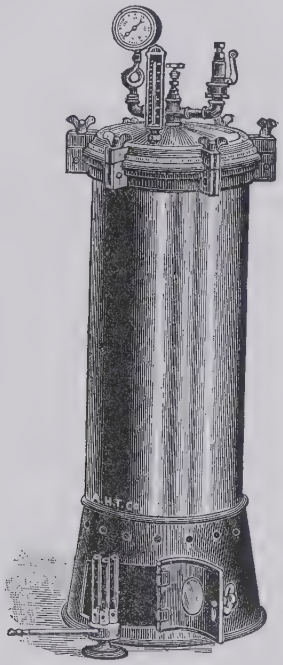


Fig. 26.—Modern autoclave.

in milk and other fluids that are injured or coagulated by exposure to 100° C. It is appropriate only when the organisms to be killed are without spores and without marked resisting powers.

Sterilization in the Autoclave.—If it should be desirable to sterilize a medium at once, not waiting the three days required by the intermittent method, it may be done by superheated steam under pressure in an autoclave (Fig.

26). Here, under a pressure of two or three atmospheres, sufficient heat is generated to immediately destroy the spores.

Because of its convenience many laboratory workers

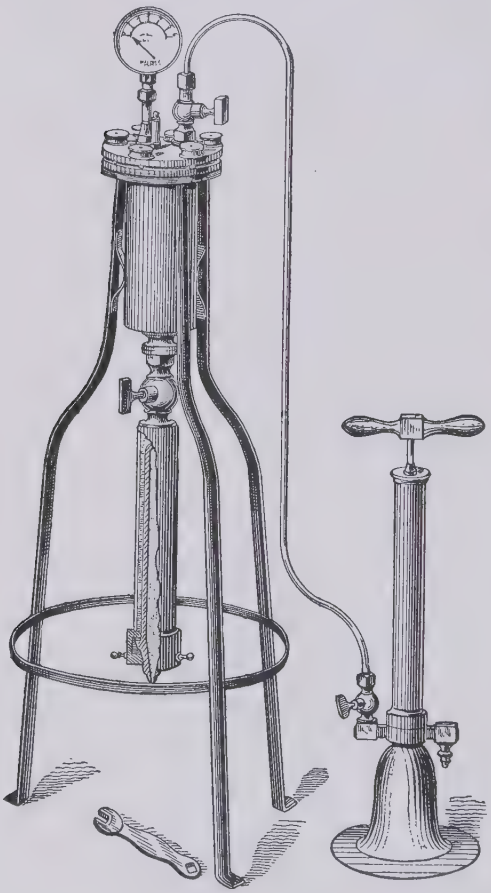


Fig. 27.—Pasteur-Chamberland filter arranged to filter under pressure.

habitually use the autoclave for the sterilization of all media not injured by the high temperature. The sterilization, to be complete, requires that the exposure shall be for fifteen minutes at 110° C. (six pounds pressure).

The media to be sterilized should be placed in the autoclave, the top firmly screwed down, but the escape-valve allowed to remain open until steam is freely generated within and replaces the hot air. The valve is then closed, and the temperature maintained for fifteen minutes or longer if the media be in bulk in flasks. The cooling must take place gradually, for if the pressure be suddenly relieved the fluids boil rapidly and the cotton plugs may be forced into the tubes or flasks by the air pressure. The chief objection to the use of the autoclave is that the high temperature brings about certain chemic changes in the media by which the reaction is altered.

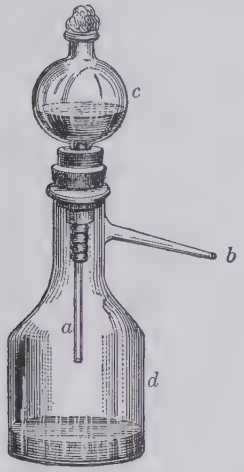


Fig. 28.—Kitasato's filter: *a*, Porcelain bougie; *b*, attachment for suction pump; *c*, reservoir; *d*, sterile receiver.

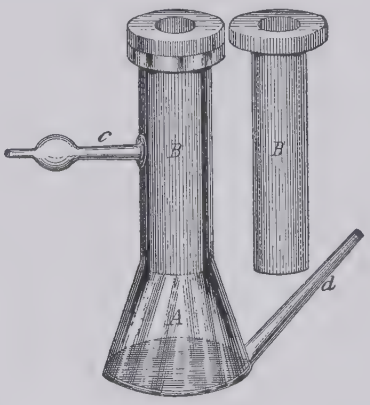


Fig. 29.—Reichel's bacteriologic filter of unglazed porcelain: *A*, Sterile receiver; *B*, porcelain filter; *c*, *d*, attachments for vacuum pump.

Sterilization by Filtration.—Liquids that cannot be subjected to heat without the loss of their most important qualities may be sterilized by filtration—*i. e.*, by passing them through unglazed porcelain or some other material whose interstices are sufficiently fine to resist the passage of bacteria. This method is largely employed for the sterilization of the unstable bacterial toxins that are destroyed by heat. Various substances have been used for filtration, as diatomaceous earth (Berkefeld filters), stone,

sand, powdered glass, etc., but experimentation has shown unglazed porcelain to be the only reliable filtering material by which to remove bacteria. Even this material, whose interstices are so small as to allow the liquid to pass through with great slowness, is only certain in its action for a time, for after it has been repeatedly used the bacteria seem able to work their way through. To be certain of the efficacy of any filter, the fluid first passed through must be tested by cultivation methods to prove that all the bacteria have been removed. The complicated Pasteur-Chamberland and the simple Kitasato and Reichel filters are shown in figures 27, 28, and 29.

The porcelain bougies as well as their attachments must be thoroughly sterilized before use.

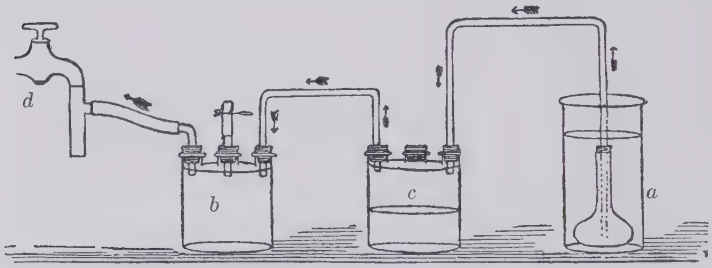


Fig. 30.—Apparatus for the rapid filtration of toxins, etc.: *a*, Filter flask; *b*, Woulff bottle to guard against regurgitation of water from the pump; *c*, reservoir for the filtrate; *d*, water vacuum pump.

After having been used, a porcelain filter must be disinfected, scrubbed, *dried thoroughly*, and then heated in a Bunsen burner or blowpipe flame until all the organic matter is consumed. In this firing process the filter first turns black as the organic matter chars, then becomes white again as it is consumed. The porcelain must be dry before entering the fire, or it is apt to crack.

It should not be forgotten that the filtrate must be received in sterile receivers and handled with care to prevent subsequent contamination.

The filtration of water, peptone solution, and bouillon is comparatively easy, but gelatin and blood-serum pass through with great difficulty, and speedily gum the filter.

A convenient apparatus used by the author for the rapid

filtration of large quantities of fluid is shown in the accompanying illustration (Fig. 30).

III. The Disinfection of Instruments, Ligatures, Sutures, the Hands, etc.—There are certain objects used by the surgeon that cannot well be rendered incandescent, exposed to dry heat at 150° C., or steamed continuously, or intermittently heated without injury. For these objects disinfection must be practised. Ever since Sir Joseph Lister introduced antisepsis, or disinfection, into surgery there has been a struggle for the supremacy of this or that highly recommended germicidal substance, with two results—viz., that a great number of feeble germicides have been discovered, and that belief in the efficacy of all germicides has been somewhat shaken; hence the *aseptic* surgery of the present day, which strives to *prevent the entrance of germs into the wound rather than their destruction after admission to it.*

For a complete discussion of the subject of antiseptics in relation to surgery the reader must be referred to text-books of surgery.

The Disinfection of the Hands, etc.—The disinfection of the skin—both the hands of the surgeon and the part about to be incised—is a matter of the utmost importance. Washing the hands with soap, which has marked germicidal properties, will in many cases suffice to destroy or remove bacteria from smooth skins. This method, which is regarded by some surgeons as adequate, is not, however, commonly regarded as sufficient protection to the patient who might be infected by any remaining micro-organisms. To overcome this, many surgeons prefer the use of sterilized gloves of thin rubber to all other means of preventing manual infections. Others prefer to use detergent and disinfectant measures. The method at present generally employed, and recommended by Welch and Hunter Robb, is as follows: The nails must be trimmed short and perfectly cleansed. The hands are washed thoroughly for ten minutes in water of as high a temperature as can comfortably be borne, soap and a previously sterilized brush being freely used, and afterward the excess of soap washed off in clean hot water. The hands are then immersed for from one to two minutes in a warm saturated solution of permanganate of potassium, then in a warm saturated solution of oxalic acid, until complete decolorization of the permanganate occurs, after which they are washed free from the acid in clean warm

water or salt solution. Finally, they are soaked for two minutes in a 1 : 500 solution of bichlorid of mercury.

Lockwood,* of St. Bartholomew's Hospital, recommends, after the use of the scissors and penknife, scrubbing the hands and arms for three minutes in hot water and soap to remove all grease and dirt. The scrubbing brush ought to be steamed or boiled before use, and kept in 1 : 1000 biniodid of mercury solution. When the soapsuds have been thoroughly washed away with plenty of clean water, the hands and arms are thoroughly washed and soaked for not less than two minutes in a solution of biniodid of mercury in methylated spirit; 1 part of the biniodid in 500 of the spirit. Hands that cannot bear 1 : 1000 bichlorid and 5 per cent. carbolic solutions bear frequent treatment with the biniodid. After the spirit and biniodid have been used for not less than two minutes, the solution is washed off in 1 : 2000 or 1 : 4000 biniodid of mercury solution.

It is a mistake to insist upon the employment of disinfecting solutions of a strength injurious to the skin. It must be obvious to every one that rough skins with numerous hangnails and fissures offer greater difficulties to be overcome in disinfection, and more readily convey micro-organisms into the wound than smooth, soft skins.

Sterilization of Ligatures, etc.—Catgut cannot be sterilized by boiling without deterioration. The present method of treatment is to dry it in a hot-air chamber and then boil it in cumol, which is afterward evaporated and the skeins preserved in sterile test-tubes or special receptacles plugged with sterile cotton. Cumol was first introduced for this purpose by Krönig, as its boiling-point is 168°–178° C., and thus sufficiently high to kill spores. The use of cumol for the sterilization of catgut has been carefully investigated by Clarke and Miller.†

Ligatures of silk and silkworm-gut are boiled in water immediately before using, or are steamed with the dressings, or placed in test-tubes plugged with cotton and steamed in the sterilizer.

Sterilization of Surgical Instruments, etc.—In most hospitals, instruments are boiled before using in a 1–2 per cent. soda (sodium carbonate, sodium bicarbonate, or sodium biborate) solution, as plain water has the disadvantage of rusting them. During the operation they are either kept in

*"Brit. Med. Jour.," July 11, 1896.

†"Bull. of the Johns Hopkins Hospital," Feb. and March, 1896.

the boiled water or in a carbolic solution, or are dried with a sterile towel. Andrews makes special mention of the fact that the instruments must be completely immersed to prevent rusting.

Disinfection of the Wound.—Cleansing solutions (normal salt solution) and disinfecting solutions (such as 1 : 10,000 to 1 : 1000 bichlorid of mercury) are only applied to septic wounds.

IV. The Disinfection of Sick-chambers, Dejecta, etc.—The Air of the Sick-room.—It is impossible to sterilize or disinfect the atmosphere of a room during its occupancy by the patient. It is entirely useless to place beneath the bed or in the corner of a room small receptacles filled with carbolic acid or chlorinated lime. These can serve no purpose for good, and may do harm by obscuring odors emanating from harmful materials that should be removed from the room. The practice is only comparable to the old faith in the virtue of asafetida tied in a corner of the handkerchief as a preventive of cholera and smallpox.

Certain fundamental principles govern the rationale of disinfection, and must be kept in mind: (1) the reagent employed should be known to act destructively upon bacteria; (2) it must be applied to the bacteria to be killed; (3) it must be applied in sufficiently concentrated form, and (4) it must be left in contact with the bacteria long enough to accomplish the effect desired.

During the period of illness the chamber in which the patient is confined should be freely ventilated. An abundance of fresh, pure air is a comfort to the patient and a protection to the doctor and nurse.

After recovery or death one should rely less upon fumigation than upon disinfection of the walls and floor, the similar disinfection of the wooden part of the furniture, and the sterilization of all else. The fumes of sulphur do some good, especially when combined with steam, but are greatly overestimated in action and are *very destructive to furnishings*, so that they are rapidly giving way to the more satisfactory, less destructive, and equally germicidal formaldehyd vapor.

Formaldehyd is probably the best germicide that has yet been recommended. Its use for the disinfection of rooms and hospital wards was first suggested by Trillat in 1895, but it did not make much stir in the medical world until a year or more had passed and a 40 per cent. solution of the gas, under the name of "Formalin," had been placed upon the

market. The original method of disinfection consisted of the evolution of the gas from methyl alcohol by volatilizing it in a steam apparatus, and passing the vapor over a heated metal plate. At present many efficient forms of apparatus are upon the market.

The gas has enormous bactericidal powers, is not injurious to man, and has no destructive effect upon furnishings.

It is not always necessary to use a special apparatus in order to disinfect with formaldehyd; one can, in an emergency, hang up a number of sheets saturated with the 40 per cent. formaldehyd solution in the room to be disinfected. The number of sheets must vary with the size of the room, as each is able to evolve but a certain amount of the gas, and the quantity necessary for disinfection varies with the size of the room. Care must also be exercised that the hands do not become wet with the concentrated formaldehyd solution, as it hardens the skin and deadens sensation.

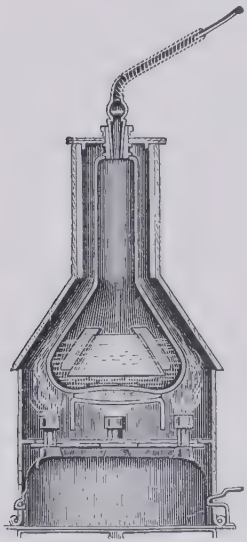


Fig. 31.—Trenner's formaldehyd gas regenerator.

The "formalin," or 40 per cent. solution of the gas, when fresh and tightly corked, is fatal to most bacteria in dilutions of from 1 : 5000 to 1 : 25,000. It can be employed with advantage to spray the walls and floors of rooms from a large atomizer, though Rosenau* finds that unless the spray discharged by the atomizer be very fine its action is uncertain. It cannot be employed upon the skin or mucous membranes, because of its marked irritating effect.

To disinfect with formaldehyd or any gaseous disinfectant, the room must be carefully closed, the cracks of the windows and doors being sealed by pasting strips of paper over them. A carefully selected apparatus is set in action, and the discharged vapor, entering the room through the keyhole or some other convenient aperture, is allowed to act undisturbed for some hours, after which the windows and doors are all thrown open to fresh air and sunlight.

* "Disinfection and Disinfectants," Philadelphia, 1902.

So far as is known at present, superficial disinfection by formaldehyd leaves nothing to be desired. Care must, however, be exercised to see that the required volume of gas is generated to disinfect the apartment. *A sufficient concentration of the gas is absolutely necessary.* The apparatus selected should also be one capable of discharging enough of the gas in a short time, as, if the evolution of the gas is too slow, polymerization is apt to occur, with the result that its disinfecting power is destroyed.

Disinfection with formaldehyd is, however, only superficial, its penetrating powers being limited. The discharge of gas into the room should only be preliminary to other and more thorough disinfection and sterilization of the contents by the application of solutions of disinfectants to the woodwork, and to the boiling of the linen, etc.

The Dejecta.—In diphtheria the expectoration and nasal

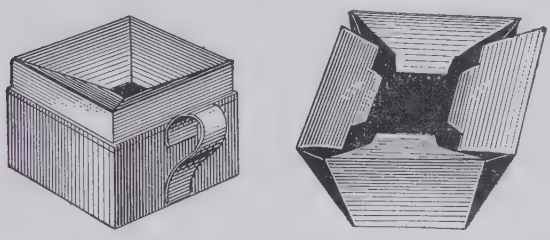


Fig. 32.—Pasteboard cup for receiving infectious sputum. When used the pasteboard can be removed from the iron frame and burned.

discharges are most important and should be received in old rags or in Japanese paper napkins—not handkerchiefs or towels—and should be burned. The sputum of tuberculous patients should either be collected in a glazed earthen vessel which can be subjected to boiling and disinfection, or, as is an excellent plan, should be received in Japanese rice-paper napkins, which can at once be burned. These napkins are not quite so good as the small pasteboard boxes (Fig. 32) recommended by some city boards of health, because, being highly absorbent, the sputum is apt to soak through and soil the fingers. For the fastidious patients in certain sanatoria, cut-glass bottles with tightly fitting lids are used to collect the sputum, and as these are not unsightly, the patients make no objection to carrying them about with them. Tuberculous patients should be provided

with rice-paper instead of handkerchiefs, and should have their towels, knives, forks, spoons, plates, etc., kept strictly apart from the others of the household and carefully sterilized after using. Patients whose mental acuity makes their sensibilities very pronounced need never be told of these arrangements.

The excreta from cases of typhoid fever and cholera require particular attention. These, and indeed all alvine matter the possible source of infection or contagion, should be received in glazed earthen vessels and immediately and intimately mixed with a 5 per cent. solution of chlorinated lime (containing 25 per cent. of chlorin) if semi-solid, or with the powder if liquid, and allowed to stand for an hour before being thrown into the drain.

Thoughtful consideration should always be given the germicides used to disinfect the discharges, lest combination of the chemical with ingredients of the discharge produce inert compounds. Thus, bichlorid of mercury cannot be used because it forms an inert compound with albumin.

The Clothing, etc.—The bed-clothing, towels, napkins, handkerchiefs, night-robes, underclothes, etc., used by a patient suffering from an infectious disease, as well as the towels, napkins, handkerchiefs, caps, aprons, and outside dresses worn by the nurse, should be regarded as infected and carefully sterilized. The only satisfactory method of doing this is by prolonged subjection to steam in a special apparatus; but, as this is only possible in hospitals, the next best thing is boiling for some time in the ordinary wash-boiler. In drying, the wash should hang longer than usual in the sun and wind. Woolen underwear can be treated exactly as if made of cotton. The woolen outer clothing of the patient, if infected, requires special treatment. Fortunately, the infection of the outer garments is unusual. The only reliable method for their sterilization is prolonged exposure to hot air at 110° C. In private practice it often becomes a grave question what shall be done with these articles. Prolonged exposure to fresh air and sunlight will, however, aid in rendering them harmless; and can be practised when it is not certain that they are actually infected. Infected articles of wool may be sent to the city hospital or to one of the moth-destroying and fumigating establishments which can be found in all large cities, and baked.

The doctor visiting a case of dangerous infection or a hospital for infectious diseases should cover his clothing with a linen or cotton gown, and protect his hair with a cap, these articles being disinfected after the visit. By such precautions he will avoid spreading infection among his patients or carrying it to his own family.

The Furniture, etc.—The destruction of infected furniture is unnecessary. The doctor treating a case of infectious disease, if he properly perform his functions, will save much trouble and money for his patient by ordering his immediate isolation in an uncarpeted, scantily and simply furnished room the moment an infectious disease is *suspected.* However, if before his removal the patient has occupied another bed, its clothing should be promptly disinfected.

After the recovery or death of the patient the walls and ceiling of the room should be sprayed with a formaldehyd solution, or the room sealed and filled with the vapor. Where this cannot be done, the walls may be rubbed with fresh bread, which Löffler has shown to be efficacious in collecting the bacteria, though very troublesome and scarcely practicable; or, if possible, the walls may be whitewashed. If they are hung with paper, they may be dampened with 1 : 1000 bichlorid of mercury solution before new paper is hung.

Strehl has demonstrated that when 10 per cent. formalin solution is sponged upon artificially infected curtains, etc., the bacteria are killed by the action of the disinfectant. This is an important adjunct to our means of disinfecting the furniture of the sick-chamber.

The floor should be scoured with 40 per cent. formaldehyd solution, 5 per cent. carbolic acid solution, or 1 : 1000 bichlorid of mercury solution (no soap being used, as it destroys the bichlorid of mercury and prevents its action), and all the wooden articles wiped off two or three times with one of the same solutions. If a straw mattress was used it should be burned and the cover boiled. If a hair mattress was used, it can be steamed or baked by the manufacturers, who usually have ovens for the purpose of destroying moths, but which answer for sterilizing closets. Curtains, shades, etc., should receive proper attention; but, of course, the greater the precautions exercised in the beginning, the fewer the articles that will need attention in the end.

The Patient, whether he live or die, may be a means of spreading the disease unless specially cared for. After convalescence the body should be bathed with a weak bichlorid of mercury solution or with a 2 per cent. carbolic acid solution, or with 25–50 per cent. alcohol, before the patient is allowed to mingle with society, and the hair should either be cut off or carefully washed with the disinfecting solution or an antiseptic soap. In desquamative diseases it seems best to have the entire body anointed with cosmolin once daily, the unguent being well rubbed in, in order to prevent the particles of epidermis, in which the specific contagium probably occurs, being distributed through the atmosphere. Carbolated may be better than plain cosmolin, not because of the very slight antiseptic value it possesses, but because it helps to allay the itching which may accompany the desquamative process.

After the patient is about the room again, common sense will prohibit the admission of visitors until the suggested disinfective measures have been adopted, and after this, touching, and especially kissing him, should be avoided for some time.

The bodies of those that die of infectious diseases should be washed in a strong disinfectant solution, and should be given a strictly private funeral. If this be impossible, the body should be sealed in the coffin and only the face viewed through a plate of glass. In my judgment, the body is best disposed of by cremation, though it seems to be erroneous to suppose that a dead body can remain for an indefinite period a source of infection. Esmarch * made a series of laboratory experiments to determine the fate of pathogenic bacteria in the dead body, and from them concludes that in septicemia, cholera, anthrax, malignant edema, tuberculosis, tetanus, and typhoid fever the pathogenic bacteria all die sooner or later, more rapidly during active decomposition than during preservation of the tissues. Lack of oxygen may also be a cause of their disappearance.

* "Zeitschrift für Hygiene," 1893.

CHAPTER VII.

CULTURE MEDIA AND THE CULTIVATION OF BACTERIA.

In order to observe them accurately the bacteria must be separated from their natural surroundings and artificially cultivated upon certain prepared media of standard composition, in such a manner that only organisms of the same kind are together. The effect of one organism upon the growth of another, by neutralizing its metabolic products, by changing the reaction of the medium in which it grows so as to inhibit further multiplication, by dissolving the other species through its enzymes, etc., suffices to show how impossible it is to determine the natural history of any organism unless it be kept strictly away from other species.

Various organic and inorganic mixtures have been suggested for the cultivation of bacteria, but few have met with particular favor and have become standards. At the present time certain standard media are used in every laboratory in the world; all our systematic study of the organisms depends upon the behavior of bacteria upon them, and no study of micro-organisms can be considered complete unless the behavior of the bacteria upon them has been carefully considered.

Our studies of the biology of the bacteria have shown that they grow best in mixtures containing at least 80 per cent. of water, of neutral or feebly alkaline reaction, and of a composition which, for the pathogenic forms at least, should approximate the juices of the animal body. It might be added that transparency is a very desirable quality, and that the most generally useful culture media are those that can be liquefied and solidified at will.

All accurate bacteriologic culture experiments require that an exact knowledge of the chemistry of the media used shall be at hand. The importance of this and the necessity for having exact information regarding the reaction of the media are well brought out in the following excerpts from

the Report of the Committee of Bacteriologists of the American Public Health Association:*

"The first thing to obtain is a standard 'indicator' which will give uniform results. These requirements are best fulfilled by phenolphthalein."

"The question of the proper reaction of media for the cultivation of bacteria and the method of obtaining this reaction have been discussed in a valuable paper by Mr. George W. Fuller, published in the 'Journal of the American Public Health Association,' Oct., 1895, vol. xx, p. 321."

"Method of determining the degree of reaction of culture media: For this most important part in the preparation of culture media, burets graduated into one-tenth c.c. and three solutions are required—

"1. A 0.5 per cent. solution of commercial phenolphthalein, in 50 per cent. alcohol.

"2. A $\frac{n}{20}$ solution of sodium hydroxid.

"3. A $\frac{n}{20}$ solution of hydric chlorid.

"Solutions 2 and 3 must be accurately made and must correspond with the normal solutions soon to be referred to.

"Solutions of sodium hydroxid are prone to deterioration from the absorption of carbon dioxid and the consequent formation of sodium carbonate. To prevent as much as possible this change, it is well to place in the bottle containing the stock solution a small amount of calcium hydroxid, while the air entering the burets or the supply bottles should be made to pass through a U-tube containing caustic soda, to extract from it the carbon dioxid."

"The medium to be tested, all ingredients being dissolved, is brought to the prescribed volume by the addition of distilled water to replace that lost by boiling, and after being thoroughly stirred, 5 c.c. are transferred to a 6-inch porcelain evaporating-dish. To this 45 c.c. of distilled water are added and the 50 c.c. of fluid are boiled for three minutes over a flame. One cubic centimeter of the solution of phenolphthalein (No. 1) is then added, and by titration with the required reagent (No. 2 or No. 3) the reaction is determined. In the majority of instances the reaction will be found to be acid, so that the $\frac{n}{20}$ sodium hydroxid is the reagent most frequently required. This determination should be made not less than three times and the average of the results obtained taken as the degree of the reaction.

"One of the most difficult things to determine in this process is exactly when the neutral point is reached as shown by the color developed, and to be able in every instance to obtain the same shade of color. To aid in this regard, it may be remarked that in bright daylight the first change that can be seen on the addition of alkali is a very faint darkening of the fluid, which, on the addition of more alkali, becomes a more evident color and develops into what might be described as an Italian pink. A still further addition of alkali suddenly develops a clear and bright pink color, and this is the reaction always to be obtained. All titrations should be made quickly and in the hot solutions to avoid complications arising from the presence of carbon dioxid.

"The next step in the process is to add to the bulk of the medium the calculated amount of the reagent, either alkali or acid, as may be

* "Jour. Amer. Public Health Assoc.," Jan., 1898, p. 72.

determined. For the purpose of neutralization a normal solution of sodium hydroxid or of hydric chlorid is used, and after being thoroughly stirred the fluid thus neutralized is again tested in the same manner as at first, to insure the proper reaction of the medium being attained. When neutralization is to be effected by the addition of an alkali, it not infrequently happens that after the calculated amount of normal solution of sodium hydroxid has been added, the second test will show that the medium is acid to phenolphthalein, to the extent sometimes of 0.5 to 1 per cent. This discrepancy is perhaps due to side reactions which are not understood. The reaction of the medium, however, must be brought to the desired point by the further addition of sodium hydroxid, and the titrations and additions of alkali must be repeated until the medium has the desired reaction (*i. e.*, 0.0 per cent. to 0.005 per cent.; see below).

"After the prescribed period of heating, it is frequently found that the medium is again slightly acid, usually about 0.5 per cent. Without correcting this, the fluid is to be filtered and the calculated amount of acid or alkali is to be added to change the reaction to the one desired. A still further change in reaction is not infrequently to be observed after sterilization, the degree of acidity varying apparently with the composition of the media and the degree and continuance of the heat."

"Manner of expressing the reaction: Since at the time the reaction is first determined culture media are more often acid than alkaline, it is proposed that acid media be designated by the plus sign and alkaline media by the minus sign, and that the degree of acidity or alkalinity be noted in parts per hundred. Thus, a medium marked +1.5 would indicate that the medium was acid, and that 1.5 per cent. of $\frac{n}{1}$ sodium hydroxid is required to make it neutral to phenolphthalein; while —1.5 would indicate that the medium was alkaline and that 1.5 per cent. of $\frac{n}{1}$ acid must be added to make it neutral to the indicator."

"*Standard reaction of media (provisional):*

"Experience seems to vary somewhat as to the optimum degree of reaction which shall be uniformly adopted in the preparation of standard culture media. To what extent this is due to variation in natural conditions as compared with variations of laboratory procedure it seems impossible to state. Somewhat different degrees of reaction for optimum growth are required, not only in or upon the media of different composition and by bacteria of different species, but also by bacteria of the same species when in different stages of vitality. The bulk of available evidence from both Europe and America points to a reaction of +1.5 as the optimum degree of reaction for bacterial development in inoculated culture media. While this experience is at variance with that in several of our own laboratories, it has been deemed wisest to adopt +1.5 as the provisional standard reaction of media, but with the recommendation that the optimum growth reaction be always recorded with the species."

Many bacteriologists regard a reaction of +1.0 as a more desirable standard and use it exclusively.

BOUILLON.

This is one of the most useful and most simple media. It can be prepared from meat or from meat extract, and is

the basis of most of the culture media. The addition of 10 per cent. of gelatin makes it "gelatin"; that of 1 per cent. of agar-agar makes it "agar-agar." The preparation of these media, however, requires special directions, which will be given below.

I. To Prepare Bouillon from Fresh Meat.—To 500 grams of finely chopped lean, boneless beef, 1000 c.c. of clean water are added and allowed to stand for about twelve hours on ice. At the end of this time the liquor is decanted, that remaining on the meat expressed through a cloth, and then, as the entire quantity is seldom regained, enough water added to bring the total amount up to 1000 c.c. This liquid is called the *meat-infusion*. To it 10 grams of Witte's or Fairchild's dried beef-peptone and 5 grams of sodium chlorid are added, and the whole boiled until the albumins of the meat-infusion coagulate, titrated or otherwise corrected for acidity, then boiled again for a short time, and then filtered through a fine filter-paper. It should be slightly yellow and perfectly clear and limpid. Smith,* referring to bouillon intended for the culture of diphtheria bacilli for toxin, says that when the peptones are added before boiling most of them are lost, and therefore recommends that the meat-infusion be boiled and filtered and the solid ingredients added and dissolved subsequently. The reaction, which is strongly acid, is then carefully corrected by titration according to the directions already given.

For rough work in students' classes litmus paper may be used as an indicator for determining and correcting the acidity resulting from the sarcolactic and other acids in the meat-infusion, the alkaline solution being added drop by drop until a faint blue appears on the red paper; or the method of using phenolphthalein can be employed, the addition of the alkaline solution being continued until a drop of the bouillon produces a red spot upon phenolphthalein paper, made, as suggested by Timpe, by saturating bibulous paper cut into strips with a solution of 5 grams of phenolphthalein to 1 liter of 50 per cent. alcohol. Acids do not change the appearance of the paper, but small traces of alkali turn it red.

If the bouillon is to be employed for exact work, these crude methods should not be adopted, but chemical titration according to the method already given should be performed.

* "Trans. Assoc. Amer. Phys.," 1896.

After titration the bouillon must again be boiled for a few minutes, in order to precipitate the acid albumins, as much water added as has been lost by evaporation, and the fluid filtered through a pharmaceutic filter.

The filtered fluid is dispensed in previously sterilized tubes with cotton plugs—about 10 c.c. to each—or in flasks, and is then sterilized by steam three successive days for fifteen to twenty minutes each, according to the directions already given for intermittent sterilization, or superheated in the autoclave.

The loss of water during boiling is an important matter to bear in mind, as unless properly replaced it is the cause of disproportion between the fluids and solids of the media. The quantity must therefore be measured before filtration and enough water added to replace what has been lost. Measuring before filtration is comparatively easy with bouillon, but difficult with heavy liquids, like the gelatin and agar-agar solutions. To overcome this difficulty it is best to make the entire preparation by weight and not by volume. A pair of platform scales with sliding indicators will first balance the empty kettle and then show the correct quantity of each added ingredient. After boiling, the kettle can be returned to the scale and the exact quantity of water to be added determined.

II. To Prepare Bouillon from Meat Extract.—When desirable, the bouillon may also be prepared from beef-extract, the method being very simple: To 1000 c.c. of clean water 10 grams of Witte's dried beef-peptone, 5 grams of sodium chlorid, and about 2 grams of beef-extract are added. The solution is boiled until the constituents are dissolved, titrated, and filtered *when cold*. If it be filtered while hot, there is always a subsequent precipitation of meat-salts, which clouds it.

Bouillon and other liquid culture media are best dispensed and kept in small receptacles—test-tubes or flasks—in order that a single contaminating organism, should it enter, may not spoil the entire quantity. A very convenient, simple apparatus used by bacteriologists for filling tubes with liquid media is shown in figure 33. It consists of a funnel to which a short glass pipet is attached by a bit of rubber tubing. A pinch-cock, at *b*, controls the outflow of the liquid. The apparatus need not be sterilized before

using, as the culture medium must subsequently be sterilized either by the intermittent method or in the autoclave after the tubes are filled. The test-tubes and flasks into which the culture medium is filled must, however, be previously sterilized by dry heat, unless the subsequent sterilization

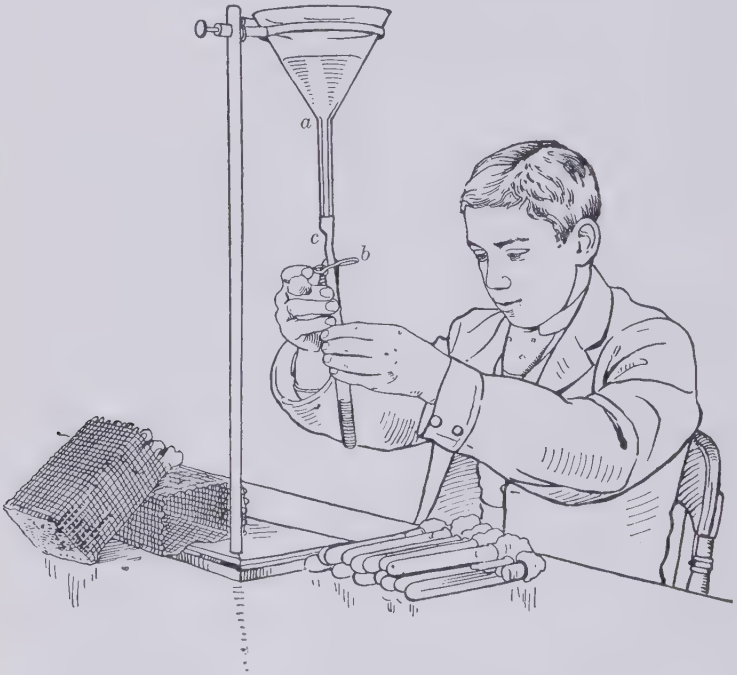


Fig. 33.—Funnel for filling tubes with culture media (Warren): *a*, Funnel containing the culture media in liquid condition; *b*, pinch-cock by which the flow of fluid into the test-tube is regulated; *c*, rubber tubing.

is to be performed in the autoclave, when it may be unnecessary.

Sugar bouillon is bouillon containing in solution known percentages of such sugars as glucose, lactose, saccharose, etc. As Smith* has pointed out, if the quantity of sugar in the bouillon is to be accurately known, it is necessary to first destroy the muscle sugars in the meat-infusion by adding a

* "Jour. of Exp. Med.," II, No. 5, p. 546.

culture of the colon bacillus to the meat-infusion and permitting fermentation to continue overnight before finishing the bouillon and adding the known quantity of whatever, sugar is desired. About 1 per cent. of dextrose, lactose, saccharose or galactose is all that is required. More may be injurious. If the bouillon be made from meat extract, fermentation may not be necessary.

The sugar bouillons should not be sterilized in the autoclave, as the high temperatures chemically alter the sugars.

GELATIN.

The culture medium known as gelatin has the decided advantage over bouillon that it is not only an excellent food for bacteria, and, like the bouillon, transparent, but also is *solid* at the room temperature. Nor is this all. It is a transparent solid that can be made liquid or solid at will. It is prepared as follows:

To 1000 c.c. of meat-infusion or to 1000 c.c. of water containing 2 grams of beef-extract in solution, 10 grams of peptone, 5 grams of salt, and 100 grams of gelatin ("Gold label" is the best commercial article) are added, and heated until the ingredients are dissolved. The solution reacts strongly acid and must be corrected by titration, as already described. It must then be returned to the fire and boiled for about an hour. As gelatin is apt to burn when boiled over the direct flame, double boilers have been suggested, but unless the outer kettle is filled with brine or saturated calcium chlorid solution, they are very slow, and when proper care is exercised there is really no great danger of the gelatin burning. It must be stirred occasionally, and the flame should be so distributed by wire gauze or by placing a sheet of asbestos between it and the kettle as not to act upon a single point. At the end of the hour the albumins of the meat-infusion will be coagulated and the gelatin thoroughly dissolved. Günther has shown that the gelatin congeals better if allowed to dissolve slowly in warm water before boiling. As much water as has been lost by vaporization during the process of boiling should be replaced. It is well to cool the liquid to about 60° C., add the water mixed with the white of an egg to clear the liquid, boil again for half an hour, and filter.

If the filter paper be of good quality, properly folded

(pharmaceutic filter), wet with boiling water, and if the gelatin be properly dissolved, the whole quantity should pass through before cooling too much. Should only half go through before cooling, the remainder must be returned to the pot, heated to boiling once more, and then passed through a new filter paper. As a matter of fact, gelatin usually filters readily. A wise precaution is to catch the first few centimeters in a test-tube and boil them, so that if cloudiness show the presence of uncoagulated albumin, the whole mass can be boiled again. The finished gelatin, which is *perfectly transparent* and of an amber color, is at once distributed into sterilized tubes and sterilized like the bouillon by the intermittent method. The sterilization can also be satisfactorily performed by the use of the autoclave at 110°–115° C. for fifteen minutes, but this method is probably less well adapted to the sterilization of gelatin than of the other media, as the high degree of heat injures its subsequent solidifying power.

Sterilized gelatin or other culture medium can be kept *en masse* indefinitely, but should a contaminating microorganism accidentally enter, the whole quantity will be spoiled; if, on the other hand, it be dispensed and kept in tubes, several of them may be contaminated without serious loss. When properly sterilized and protected, it should keep indefinitely.

AGAR-AGAR.

Agar-agar is the commercial name of a preparation made from a Ceylonese sea-weed. It reaches the market in the form of long shreds of semi-transparent, isinglass-like material, less commonly in long bars of compressed flakes, rarely in the form of powder. It dissolves slowly in boiling water with a resulting thick jelly when cold. The jelly, which solidifies between 40° and 50° C., cannot again be melted except by the elevation of its temperature to the boiling-point. The culture medium made from agar-agar is nearly transparent, and is almost as useful as gelatin, as in addition to its ability to liquefy and solidify, it has the decided advantage of remaining solid at comparatively high temperatures so as to permit keeping the cultures grown upon it at the incubation temperature,—*i. e.*, 37° C.,—at which temperature gelatin is always liquid.

The preparation of agar-agar is commonly described in

the text-books as one "requiring considerable patience and much waste of filter paper." In reality, it is not difficult if a good heavy filter paper be obtained and no attempt made to filter the solution until the agar-agar is perfectly dissolved.

It is prepared as follows: To 1000 c.c. of bouillon made as described above, preferably of meat instead of beef-extract, 10 to 15 grams of agar-agar are added. The mixture is boiled vigorously for an hour in an open pot over the direct gas flame or in the double boiler with saturated calcium chlorid solution in the outside pot. After being cooled to about 60° C., and after the correction of the reaction by titration, an egg beaten up in water is added, and the liquid again boiled until the egg-albumen is entirely coagulated.

After the second boiling and the replacement of the volatilized water, the agar-agar is filtered through a carefully folded pharmaceutic filter wet with boiling water. It may expedite matters to pour in about one-half of the solution, keep the remainder hot, and subsequently add it when necessary.

The formerly much employed hot-water and gas-jet filters are unnecessary. If properly prepared, the whole quantity will filter in from fifteen to thirty minutes.

Ravenel * prepares agar-agar by making two solutions, one representing the meat-infusion, but twice the usual strength, the other the agar-agar dissolved in one-half the usual quantity of water. The agar-agar is dissolved by exposure to superheated steam in the autoclave, after which the two solutions are poured together and boiled until all of the albumins are precipitated. The coagulation of the albumins of the meat-infusion serves to clarify the agar-agar.

If agar-agar is to be made with beef-extract, the bouillon should be made first and filtered *when cold*, to exclude the uratic salts which otherwise precipitate in the agar-agar when cold and form an unsightly cloud.

The finished agar-agar should be a colorless, nearly transparent, firm jelly. It is dispensed in tubes like the gelatin and bouillon, sterilized by steam, either by the intermittent process or in the autoclave, and after the last sterilization, before cooling, each tube is inclined against a slight elevation, so as to permit the jelly to solidify obliquely and afford an extensive flat surface for the culture.

* "Journal of Applied Microscopy," June, 1898, vol. 1, No. 6, p. 106.

After the agar-agar jelly solidifies it retracts so that a little water collects at the lower part of the tube. This should not be removed, as it keeps the jelly moist, and also distinctly influences the character of the growth of the bacteria.

Glycerin Agar-agar.—For an unknown reason certain bacteria that will not grow upon agar-agar prepared as described will do so if 3 to 7 per cent. of glycerin be added after filtration. Among these is the tubercle bacillus, which, not growing at all upon plain agar-agar, will grow well when glycerin is added—a fact discovered by Roux and Nocard. The glycerin added to bouillon or any other medium has the same advantageous influence.

Blood Agar-agar was recommended by R. Pfeiffer for the cultivation of the influenza bacillus. It is ordinary agar-agar whose surface is coated with a little blood secured under antiseptic precautions from the finger-tip, ear-lobule, etc., of man, or from the vein of one of the lower animals. Some bacteriologists prepare a hemoglobin agar-agar by spreading a little powdered hemoglobin upon the surface of the agar-agar. This has the disadvantage that powdered hemoglobin is not sterile, and the medium must be again sterilized after its addition.

The blood agar-agar should be kept in the incubator a day or two before use so as to insure perfect sterility.

BLOOD-SERUM.

The great advantage possessed by this medium is that it is itself a constituent of the body, and hence offers opportunities for the development of the parasitic forms of bacteria. If the blood-serum is to be employed fresh, it must either be heated or kept sufficiently long to lose its natural germicidal properties. The statement that serum represents the normal body-juice is erroneous, as it is minus the fibrin factors and some of the salts, and contains new bodies liberated from the destroyed leukocytes. Solidified blood-serum, exposed to the heat of the sterilizing apparatus, in no sense resembles the body-juices.

It is one of the most difficult media to prepare. The blood must be obtained either by bleeding some good-sized animal or from a slaughter-house in appropriate receptacles, the best things for the purpose being 1-quart fruit jars with tightly fitting lids. The jars are sterilized by heat, closed,

and carried to the slaughter-house, where the blood is permitted to flow into them from the severed vessels of the animal. It seems advisable to allow the first blood to escape, as it is likely to become contaminated from the hair. By waiting until a coagulum forms upon the hair the danger of contamination is obviated. The jars, when full, are allowed to stand undisturbed until firm coagula form within them, after which they are carried to the laboratory and stood upon ice for forty-eight hours, by which time the clots will have retracted considerably, and a moderate amount of clear serum can be removed by sterile pipets and placed in sterile tubes. If the serum obtained be red and clouded from the presence of corpuscles, it may be pipetted into sterile cylinders and allowed to sediment for twelve hours, then repipetted into tubes. It is evident that such frequent manipulations afford numerous chances of infection; hence the sterilization of the serum becomes of the greatest importance.

As the demand for serum has been considerable during the last few years, commercial houses dealing in biologic products now market fresh horse serum, preserved with chloroform, in liter bottles. This can be employed with great satisfaction, the chloroform being driven off during coagulation and sterilization.

If it be desirable to use the serum as a liquid medium, it is exposed to a temperature of 60° C. for one hour upon each of five consecutive days. To coagulate the serum and make a solid culture medium, it may be exposed twice, for an hour each time—or three times if there be reason to think it badly contaminated—to a temperature just short of the boiling-point. During the process of coagulation the tubes should be inclined, so as to offer an oblique surface for the growth of the organisms. The serum thus prepared should be white, but may have a reddish-gray color if many red corpuscles be present. It is always opaque and cannot be melted; once solid, it remains so.

Koch devised a special apparatus (Fig. 34) for coagulating blood-serum. The bottom should be covered with cotton, a single layer of tubes placed upon it, the glass lid closed and covered with a layer of felt, and the temperature elevated until coagulation occurs. The repeated sterilizations may be conducted in this same apparatus, or may be done equally well in a steam apparatus, the cover of which is not completely closed, for if the temperature of the serum be raised

too rapidly it is certain to bubble, so that the desirable smooth surface upon which the culture is to be made is ruined.

Like other culture media, blood-serum and its combinations may be sterilized in the autoclave and much time thus saved. The serum should, however, first be coagulated, else bubbling is apt to occur and ruin its surface. The autoclave temperature unfortunately makes the preparation very firm and hard, considerable fluid being pressed out of it.

It is said that considerable advantage is secured from the addition of *neutrose* to blood-serum, which prevents its coag-

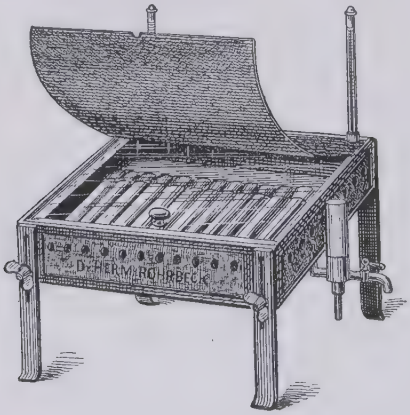


Fig. 34.—Koch's apparatus for coagulating and sterilizing blood-serum.

ulating when heated. It can then be sterilized like bouillon and can subsequently be solidified, when desired, by the addition of some agar-agar.

Fresh blood-serum can be kept on hand in the laboratory, in sterile bottles, by adding an excess of chloroform. In the process of coagulation and sterilization the chloroform is evaporated ; the serum is unchanged by its presence.

Löffler's Blood-serum Mixture, which seems rather better for the cultivation of some species than the blood-serum itself, consists of 1 part of a beef-infusion bouillon containing 1 per cent. of glucose and 3 parts of liquid blood-serum. After being well mixed the fluid is distributed in

tubes, and sterilized and coagulated like the blood-serum itself. As prepared by Löffler it was soft, semi-gelatinous and semi-transparent, not firm and white; therefore should be sterilized at low temperatures. Many organisms grow more luxuriantly upon it than upon either plain blood-serum or other culture media. Its especial usefulness is for the cultivation of Bacillus diphtheriæ, which grows upon it rapidly and with a characteristic appearance.

Alkaline Blood-serum.—According to Lorrain Smith, a very useful culture medium can be prepared as follows: To each 100 c.c. of blood-serum add 1–1.5 c.c. of a 10 per cent. solution of sodium hydrate and shake it gently. Put sufficient of the mixture into each of a series of test-tubes, and, laying them upon their sides, sterilize like blood-serum, taking care that their contents are not heated too quickly, as then bubbles are apt to form. The result should be a clear, solid medium consisting chiefly of alkali-albumins. It is especially useful for Bacillus diphtheriæ.

Deycke's Alkali-albuminate.—One thousand grams of meat are macerated for twenty-four hours with 1200 c.c. of a 3 per cent. solution of potassium hydrate. The clear brown fluid is filtered off and pure hydrochloric acid carefully added while a precipitate forms. The precipitated albuminate is collected upon a cloth filter, mixed with a small quantity of liquid, and made distinctly alkaline. To make solutions of definite strength it can be dried, pulverized, and redissolved.

The most useful formula used by Deycke was a 2.5 per cent. solution of the alkali-albuminate with the addition of 1 per cent. of peptone, 1 per cent. of NaCl, and gelatin or agar-agar enough to make it solid.

Potatoes.—Without taking time to review the old method of boiling potatoes, opening them with sterile knives, and protecting them in the moist chamber, or the much more easily conducted method of Esmarch in which the slices of potato are sterilized in the small dishes in which they are afterward kept and used, we will at once pass to what seems the most simple and satisfactory method—that of Bolton and Globig.*

With the aid of a cork-borer or Ravenel potato cutter (Fig. 35) a little smaller in diameter than the test-tube ordinarily used, a number of cylinders are cut from potatoes.

* "The Medical News," vol. L, 1887, p. 138.

Rather large potatoes should be used, the cylinders being cut transversely, so that a number, each about an inch and a half in length, can be cut from one potato. The skin is removed from the cylinders by cutting off the ends, after which each cylinder is cut in two by an oblique incision, so as to leave a broad, flat surface. The half-cylinders are placed each in a test-tube previously sterilized, and are exposed three times, for half an hour each, to the streaming steam of the sterilizer. This steaming cooks the potato and also sterilizes it. Such potato cylinders are apt to deteriorate rapidly, first by turning very dark, second by drying so as to be useless. Abbott has shown that if the cut cylinders be allowed to stand for twelve hours in running water before being dispensed in the tubes, they are not so apt to turn dark. Drying may also be prevented by adding a few drops of clean water to each tube before sterilizing. Some workers insert a bit of glass or a pledget of glass wool into the bottom of the tube so as to support the potato and keep it up out of the water. It is not necessary to have a special small chamber blown in the tube to contain this water, only a small quantity of which need be added. The special reservoir increases the trouble of cleaning the tubes.

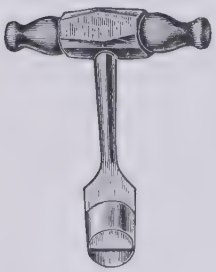


Fig. 35.—Ravenel's potato cutter.

If the work to be done with potatoes must be accurate, it may be necessary to correct their variable reaction, especially if the acids have not been sufficiently removed by the washing in running water already described.

To do this the cut cylinders are placed in a measured quantity of distilled water and steamed for about an hour. The reaction of the water is then determined by titration and the desired amount of sodium hydroxid added to correct the reaction, after which the potatoes are steamed in the corrected solution for about thirty minutes before being placed in the tubes.

A *potato-juice* has also been suggested, and is of some value. It is made thus: To 300 c.c. of water 100 grams of grated potato are added, and allowed to stand on ice over night. Of the pulp, 300 c.c. are expressed through a cloth and cooked for an hour on a water-bath. After cooling the

liquid is filtered, titrated if desired, and receives an addition of 4 per cent. of glycerin. Upon this medium the tubercle bacillus grows well, especially when the reaction of the medium is acid.

Milk.—Milk is a useful culture medium. As the cream which rises to the top is a source of inconvenience, it is best to secure fresh milk from which the cream has been removed by a centrifugal machine. It is given the desired degree of alkalinity by titration, dispensed in sterile tubes, and sterilized by steam by the intermittent method or in the autoclave. The opaque nature of this culture medium often permits the undetected development of contaminating organisms. A careful watch should therefore be kept lest it spoil.

Litmus Milk.—This is milk to which just enough of a saturated watery solution of pulverized litmus is added to give a distinct blue color after titration. Litmus milk is probably the best reagent for determining acid and alkali production by bacteria.

The watery solution of litmus, being a vegetable infusion, is likely to be spoiled by micro-organismal growth, hence must be treated like the culture media and sterilized by steam every time the receptacle in which it is kept is opened.

If litmus be added to the milk before sterilization, it is apt to be browned or decolorized, so that it is better to sterilize the two separately and pour them together subsequently. It is said that lacmoid is never thus changed, and many workers prefer it to litmus on that account.

Petruschky's Whey.—In order to differentiate between acid and alkali producers among the bacteria, Petruschky has recommended a neutral whey colored with litmus. It is made as follows:

To a liter of fresh skimmed milk 1 liter of water is added. The mixture is violently shaken. About 10 c.c. are taken out as a sample to determine how much hydrochloric acid must be added to produce coagulation of the milk, and, having determined the least quantity required for the whole bulk, it is added. After coagulation the whey is filtered off, exactly neutralized, and boiled. After boiling it is found clouded and acid in reaction. It is therefore filtered again, and again neutralized. Litmus is finally added to the neutral liquid, so that it has a violet color, changed to blue or red by alkalies or acids.

The medium is a very useful aid in differentiating the typhoid and colon bacilli, showing well the alkali formation of the former and acid of the latter.

Peptone Solution, or Dunham's solution, is useful for the detection of certain faint colors. It is a perfectly clear, colorless solution, made as follows:

Sodium chlorid	0.5
Witte's dried peptone	1.0
Water	100.0

Boil until the ingredients dissolve; filter, fill into tubes and sterilize.

It was for a long time used for the detection of indol. A very important fact in regard to peptone has been pointed out by Garini,* who found that many of the peptones upon the market were impure, and on this account failed to show the indol reaction in cultures of bacteria known to produce it. He recommends testing the peptone to be employed by the use of the biuret reaction. The reagent used is Fehling's copper solution, with which pure peptone strikes a violet color not destroyed upon boiling, while impure peptone gives a red or reddish-yellow precipitate. Both the peptone and copper solution should be in a dilute form to make successful tests. The addition of 4 c.c. of the following solution—

Rosolic acid	0.5
Eighty per cent. alcohol	100.0

makes the peptone solution a reagent for the detection of acids and alkalies. The solution is of a pale rose color. If the organisms cultivated produce acids, the color fades; if alkalies, it intensifies. As the color of rosolic acid is destroyed by glucose, it cannot be used in culture media containing it.

Theobald Smith† has called attention to the fact that many bacteria fail to grow in Dunham's solution, and recommends that, for the detection of indol, bouillon free of dextrose be used instead. All bacteria grow well in it, and the indol reaction is pronounced in sixteen-hour-old cultures. His method of preparation is as follows: Beef-infusion, prepared either by extracting in the cold or at 60° C., is inoculated in the evening with a rich fluid culture of some acid-

* "Centralbl. f. Bakt. u. Parasitenk.," XIII, p. 790.

† "Journal of Exp. Medicine," Sept. 5, 1897, VI, p. 546.

producing bacterium (Bacillus coli) and placed in the thermostat. Early next morning the infusion, covered with a thin layer of froth, is boiled, filtered, peptone and salt added, and the neutralization and sterilization carried on as usual.

Other culture media employed for special purposes will be mentioned as occasion arises.

CHAPTER VIII.

CULTURES, AND THEIR STUDY.

THE purposes for which culture media are prepared are numerous. Through their aid it is possible to separate—or, rather, to *isolate*—bacteria, to keep them in healthy growth for considerable lengths of time, during which their biologic peculiarities can be observed and their metabolic products collected, and to introduce them free from contamination into the bodies of experiment animals.

The isolation of bacteria was next to impossible until the fluid media of the early observers were replaced by the solid culture media introduced by Koch, and exceedingly difficult until he devised the well-known "plate cultures."

A growth of artificially planted micro-organisms is called a *culture*. If such a growth contains but one kind of organism, it is known as a *pure culture*.

It has at present become the custom to use the term "culture" rather loosely, so that it does not always signify an artificially planted growth of micro-organisms, but may signify a growth taking place under natural conditions; thus, typhoid bacilli are said to occur in "pure culture" in the spleens of patients dead of that disease, because no other bacteria are associated with them; and sometimes, when the tubercle bacilli are very numerous and unmixed with other bacteria, in the expectorated fragments of cheesy matter from tuberculosis pulmonalis, they are said to occur in "pure culture."

The culture manipulations are performed either with a sterilized platinum wire or with a capillary pipet of glass.

The platinum wire is so limber that it is scarcely to be recommended, and a wire composed of platinum and iridium, which is elastic in quality, is to be preferred. The wires are about 5 cm. in length, of various thicknesses according to the use for which they are employed, and are usually fused into a thin glass rod about 17 cm. in length (Fig. 36). The wires may be straight or provided with a small loop at the end so as to conveniently take up small drops of fluid.

Heavy wires used for securing diseased tissue from animals may be flattened at the ends by hammering, and may thus be fashioned into miniature knives, scrapers, harpoons, etc., as desired.

Ravenel has invented a convenient form for carrying in the pocket. It consists of the platinum wire fastened in a heavier aluminium wire which in turn fits into a piece of glass tubing. When carried in the pocket, the position of

Fig. 36.—Platinum needles for transferring bacteria; made from No. 27 platinum wire inserted in glass rods.

the platinum wire is reversed in the glass tubing and protected by it (Fig 37).

Immediately before and immediately after use, the platinum wire is to be sterilized by heating to incandescence in a flame, in order that it convey nothing undesirable into the culture, and in order that it scatter no micro-organisms about the laboratory.

The capillary glass tubes are employed by the French for many of the manipulations. They are made of $\frac{1}{4}$- or $\frac{3}{8}$-inch glass tubing cut into 25 cm. lengths, heated at the center,

Fig. 37.—Platinum wires for bacteriologic use.

and drawn out to capillary ends about 5 cm. long. They are sealed at one end and plugged with cotton at the other, and a number of them, prepared at the same time, sterilized (Fig. 38). They can be used for all the purposes for which the platinum wire is employed, and in addition can be used as containers for small quantities of fluids sealed in them. When about to use such a tube, its sealed capillary end should be broken off with forceps, and the tube sterilized by flaming.

Technic of Culture Manipulation.—In order that accurate results may accrue from the employment of culture

media, and that cultures planted in them may not be contaminated through improper technic, it is important habitually to practise certain manipulations by which as much latitude can be given the operator as is consistent with thorough defense against contaminating organisms. To this end the containers of stored culture media should be kept in an upright position, that the cotton stoppers are not moistened or soiled. If moistened with the culture media, molds whose spores fall upon the surface of the stoppers may gradually work their mycelial threads between the fibers until they appear upon the inner surface and drop newly formed spores into the contained media, or the cotton stoppers may be glued fast and further successful manipulations prevented.

In handling tubes care must be taken to stand them up in tumblers, racks, or other contrivances, and not lay them upon the table so that the contents touch the stoppers.

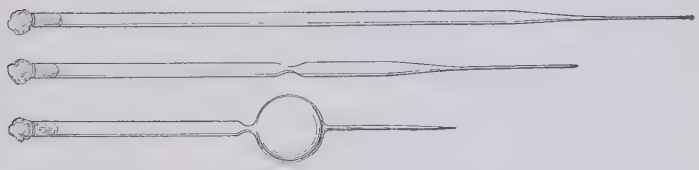


Fig. 38.—Capillary glass tubes.

When the cotton plugs are removed in order that the contents of the tubes or flasks may be inoculated or otherwise manipulated the removal and replacement should be done as quickly as convenient, and the mouth of the tube should be flamed before removal. They must be held between the fingers, by that part which projects above the glass, not laid upon the table, from which dust, and incidentally bacteria, may be taken up and subsequently dropped into the medium; nor must they be touched with the fingers at that part which enters the neck of the container lest they take up micro-organisms from the skin. The stoppers thus require careful consideration lest they become the source of future contamination.

So soon as the cotton stopper is removed, the medium is left without protection from whatever micro-organisms happen to be in the air, so that it should be replaced as soon as possible, and every manipulation requiring its removal performed expeditiously. During the time the

stopper is withdrawn it is wise to hold the tubes or other containers in an oblique or horizontal position that will aid in excluding the micro-organisms of the air. Thus, a tube held vertically can probably more easily receive such organisms than one held horizontally or reversed. Some bacteriologists make inoculations with the tubes reversed in all cases in which solid media are employed, but it is not at all necessary. If the tubes are held *obliquely*, the danger of contamination is reduced to a minimum. It is well to adopt some method of handling the tubes that has given satisfaction to others and is found convenient to one's self and habitually practise it until it becomes second nature and can be done without thought.

The usual method of making a transplantation of bacteria from culture-tube to culture-tube is, in detail, as follows:

Fig. 39.—Method of holding tubes during inoculation.

In order that any bacteria loosely scattered over the surface of the cotton stopper, and upon the glass near the mouth of the tube, may be destroyed and prevented from entering the medium as the stopper is withdrawn, both the tube containing the culture and the fresh tube to which it is to be transferred should be held for a moment in a flame and rolled from side to side so that all parts are flamed. The cotton ignites and blazes actively, but the flame can be extinguished by forcibly blowing upon it and any smoldering remains extinguished by pinching with the fingers. The tubes are now placed side by side between the thumb and upward-directed palm of the left hand, the stoppers toward the operator. The position of the tubes should be such as to permit one to see the contained media without the fingers being in the way. The stopper of the tube toward the left is removed by a gentle twist and placed between the index and middle fingers of the left hand; the stopper of the next tube similarly removed and placed between the

middle and ring fingers of the same hand (Fig. 39). If three or four tubes are to be held, the third stopper can be placed between the ring and little fingers of the left hand and the fourth retained in the right hand. The part of each stopper that enters the tube must not be touched.

The necessary manipulation is usually made with the platinum wire, which is sterilized by heating to incandescence before using. The wire must not be used while hot, but cools in a moment or two. The culture is touched, the wire entering and exiting without touching the tube, and the bacteria adhering to the wire are applied to the medium in the other tube, the same care being exerted not to have the platinum wire touch the glass. After the transfer is made, the wire is made incandescent in the flame before being returned to the table or stand made to hold it, and the stoppers returned one after the other, each to its own tube, that part entering the tube not being touched. Each stopper is given a twist as it enters the mouth of the tube.

Modifications of these directions can be made to suit the different forms of containers used, but the essential features must be maintained.

When any manipulation requires that a tube or flask be permitted to remain open an unusual length of time, its contamination from the air can be prevented for some minutes by heating its neck quite hot. The air about it, being heated by the hot glass, ascends, forming a current that carries the bacteria away from, rather than into, the receptacle.

Isolation of Bacteria.—Three principal methods are, at present, employed for securing pure cultures of bacteria. Before beginning a description of them it is well to observe that the peculiarities of certain pathogenic micro-organisms enable us to use special means for their isolation, and that these general methods are chiefly useful for the isolation of non-pathogenic organisms.

Plate Cultures.—All the methods depend upon the observation of Koch, that when bacteria are equally distributed throughout some liquefied nutrient medium that is subsequently solidified in a thin layer, they grow in scattered groups or families, called *colonies*, distinctly isolated from one another and susceptible of transplantation.

The plate cultures, as originally made by Koch, require considerable apparatus, and of late years have given place to the more ready methods of Petri and von Esmarch.

So great is their historic interest, however, that it would be a great omission not to describe the original method in detail.

Apparatus.—Half a dozen glass plates, measuring about 6 by 4 inches, free from bubbles and scratches and ground at the edges, are carefully cleaned, placed in a sheet-iron box made to receive them, and sterilized in the hot-air closet. The box is kept tightly closed, and in it the sterilized plates can be kept indefinitely before use.

A moist chamber, or double dish, about 10 inches in diameter and 3 inches deep, the upper half being just enough larger than the lower to allow it to close over it, is carefully washed. A sheet of bibulous paper is placed in the bottom, so that some moisture can be retained, and a 1 : 1000 bichlorid of mercury solution poured in and brought in contact with the sides, top, and bottom by turning the dish in all directions. The solution is emptied out, and the dish, which is kept closed, is ready for use.

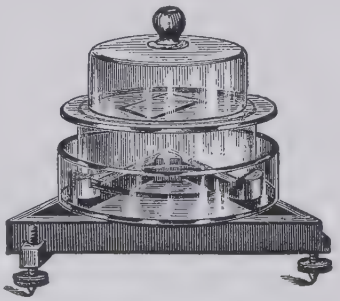


Fig. 40.—Complete leveling apparatus for pouring plate cultures, as taught by Koch.

A leveling apparatus is required (Fig. 40). It consists of a wooden tripod with adjustable screws, and a glass dish covered by a flat plate of glass upon which a low bell-jar stands. The glass dish is filled with broken ice and water, covered with the glass plate, and then exactly leveled by adjusting the screws under the legs of the tripod. When level, the cover is placed upon it, and it is ready for use.

Method.—A sterile platinum loop is dipped into the material to be examined, a small quantity secured, and stirred about so as to distribute it evenly throughout the contents of a tube of melted gelatin. If the material under examination be very rich in bacteria, one loopful may contain a million individuals, which, if spread out in a thin layer, would develop so many colonies that it would be impossible to see any one clearly; hence further dilution becomes necessary. From the first tube, therefore, a loopful of gelatin is carried to a second and stirred well, so as to distribute the organisms evenly throughout its contents.

In this tube we may have no more than ten thousand organisms, and if the same method of dilution be used again, the third tube may have only a few hundreds, and a fourth only a few dozen colonies.

After the tubes are thus inoculated, one of the sterile glass plates is caught by its edges, removed from the iron box, and placed beneath the bell-glass upon the cold plate covering the ice-water of the leveling apparatus. The plug of cotton closing the mouth of tube No. 1 is removed, and to prevent contamination during the outflow of the gelatin the mouth of the tube is held in the flame of a Bunsen burner for a moment or two. The gelatin is then cautiously poured out upon the plate, the mouth of the tube, as well as the plate, being covered by the bell-glass to prevent contamination by germs in the air. The apparatus being level, the gelatin spreads out in an even, thin layer, and, the plate being cooled by the ice beneath, it immediately solidifies, and in a few moments can be removed to the moist chamber prepared to receive it.

Fig. 41.—Glass bench.

As soon as plate No. 1. is prepared, the contents of tube No. 2 are poured upon plate No. 2, allowed to spread out and solidify, and then superimposed on plate No. 1 in the moist chamber, being separated from the plate already in the chamber by small glass benches (Fig. 41) made for the purpose and previously sterilized. After the contents of all the tubes are thus distributed, the moist chamber and its contents are stood away to permit the bacteria to grow. Where each organism falls a colony develops, and the success of the whole method depends upon the isolation of a colony and its transfer to a tube of new sterile culture media, where it can grow unmixed and undisturbed.

From the description it must be evident that only those culture media that can be melted and solidified at will can be used for plate cultures—viz., gelatin, agar-agar, and glycerin agar-agar. Blood-serum and Löffler's mixture are entirely inappropriate.

The chief drawbacks to this excellent method are the cumbersome apparatus required and the comparative impossibility of making plate cultures, as is often desirable, in the clinic, at the bedside, or elsewhere than in the laboratory. The method therefore soon underwent modifications,

the most important being that of Petri, who invented special dishes to be used instead of plates.

Petri's Dishes.—These are small glass dishes (Fig. 42), about 4 inches in diameter and ½ inch deep, with accurately fitting lids. They greatly simplify bacteriologic technic by dispensing with the plates and plate-boxes, the moist chambers and benches, and usually with the leveling apparatus

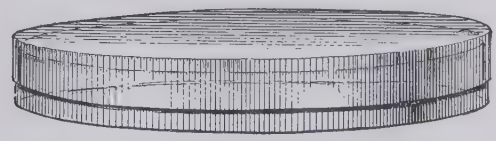


Fig. 42.—Petri dish for making plate cultures.

of Koch, though this is still employed in some laboratories, and must always be employed when an even distribution of the colonies is necessary in order that they can be accurately counted.

The method of using the Petri dishes is very simple. They are carefully cleaned, polished, and sterilized by hot air, care being taken that they are placed in the hot-air closet right side up, and after sterilization are kept covered and in that

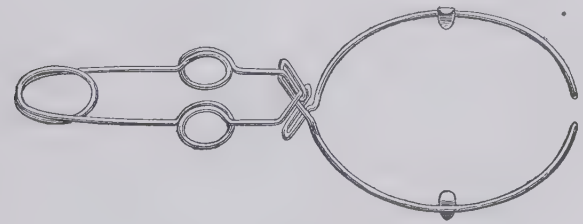


Fig. 43.—Petri dish forceps.

position. They should be sterilized immediately before using, or if they must be kept for a time should be wrapped in tissue paper and then sterilized. The tissue paper protects the interior from the accidental entrance of dust between dish and lid, keeps the dish closed, and need not be removed until the last moment before using.

Time can be saved by sterilizing the dish and cover in the direct flame, instead of in the hot-air closet, special

forceps (Fig. 43) adapted to holding them having been devised by Rosenberger.*

The dilution of the material under examination is made with gelatin or agar-agar tubes in the manner above described, the plug is removed, the mouth of the tube cautiously held for a moment in the flame, and the contents poured into one of the sterile dishes, whose lid is just sufficiently elevated to permit the mouth of the tube to enter. The gelatin is spread over the bottom of the dish in an even layer, allowed to solidify, labeled, and stood away for the colonies to develop.

Among the other advantages of the Petri dish is the convenience with which colonies can be studied with a low power lens. To do this with the Koch plates meant to remove them from the sterile chamber to the stage of a microscope and so expose them to the air, and to contamination, but to examine colonies in the Petri dish, one simply inverts it without opening and examines through the thin glass of the bottom dish without any exposure to contaminating organisms.

Esmarch's Tubes.—This method, devised by Esmarch, converts the wall of the test-tube into the plate and dispenses

Fig. 44.—Esmarch tube on block of ice (redrawn after Abbott).

with all other apparatus. The tubes, which are inoculated and in which the dilutions are made, should contain less than half the usual amount of gelatin or agar-agar. After inoculation the cotton plugs are pushed into the tubes until even

* "Phila. Med. Jour.," Oct. 20, 1900, vol. VI, No. 16, p. 760.

with their mouths, and then covered with a rubber cap, which protects them from wetting. A groove is next cut in a block of ice, and the tube, held almost horizontally, is rolled in this until the entire surface of the glass is covered with a thin layer of the solidified medium (Fig. 44). Thus the tube itself becomes the plate upon which the colonies develop.

In carrying out Esmarch's method, the tube must not contain too much of the culture medium, or it cannot be rolled into an even layer; the contents should not touch the cotton plug, lest it be glued to the glass and its subsequent usefulness injured, and no water must be admitted from the melted ice.

Colonies.—The progeny of each bacterium form a mass

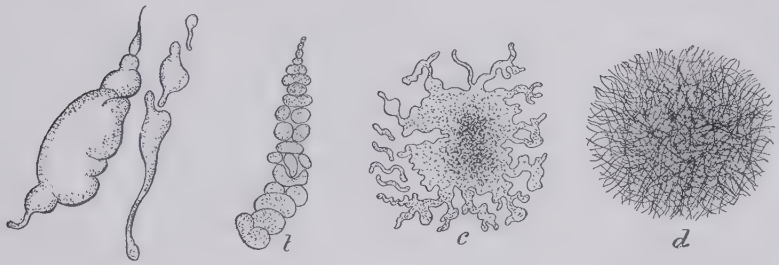


Fig. 45.—Types of colonies: *a*, Cochleate (B. coli, abnormal form); *b*, conglomerate (B. zopfii); *c*, ameboid (B. vulgatus); *d*, filamentous (Frost).

Fig. 46.—Surface elevations of growths: *a*, Flat; *b*, raised; *c*, convex; *d*, pulvinate; *e*, capitate; *f*, umbilicate; *g*, umbonate (Frost).

which is known as a *colony*. When these are separated from one another, each is spoken of as a single colony, and different characteristics belonging to different micro-organisms enables us at times to recognize by macroscopic and microscopic study of the colony the particular kind of micro-organism from which it has grown. The foregoing illustrations show the various types of colonies and the legends the terms used in describing them (Fig. 45).

Growing colonies should be observed from day to day, as it not infrequently happens that unexpected changes, such as pigmentation and liquefaction, develop after the colony

is several days old, and indeed sometimes not until much later. Again, many colonies make their first appearance as minute, sharply circumscribed points, and later spread upon the surface of the culture medium, either in the form of a thin, homogeneous layer or a filamentous cluster. It is particularly important that in describing new species of bacteria an account of the appearance of the colonies from day to day, comparing all of their variations for at least two weeks, should be included.

Pure Cultures.—Single colonies also subserve a second very important purpose, that of enabling us to secure pure cultures of bacteria from a mixture. The usual method of doing this is by the use of plates, Petri dishes, or Esmarch tubes according to the methods already described. For

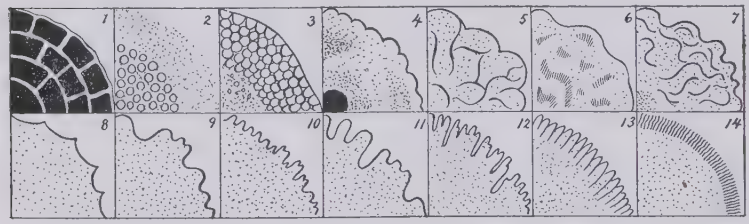


Fig. 47.—Microscopic structure of colonies: 1, Areolate; 2, grumose; 3, moruloid; 4, clouded; 5, gyrose; 6, marmorated; 7, reticulate, 8, repand; 9, lobate; 10, erose; 11, auriculate; 12, lacerate; 13, fimbricate; 14, ciliate (Frost).

this purpose an isolated colony is selected and carefully examined to see that it is single and not a mixture of two closely approximated colonies of different kinds, and then transplanted to a tube of an appropriate culture medium. If the colonies are few and of good size, each is transplanted to the medium directly and without instrumental assistance. If, however, the colonies are numerous, of small size, and close together, it may be necessary to do it under a dissecting microscope or even a low power of the ordinary bacteriologic microscope. This operation of transplantation is familiarly known as *fishing*.

Fishing.—It requires considerable practice and skill to fish successfully, and the student should early begin to practise it. The colony to be transplanted, selected because of its isolation, its typical appearance, and convenient posi-

tion on the plate, is brought to the center of the field and the plate firmly held in position with the left hand. A sterile platinum wire is held in the right hand, the little finger, comfortably fixed upon the stage of the microscope, being used to support the hand. As the operator looks into the microscope the point of the platinum wire is carefully brought into the field of vision without touching either the lens of the microscope or any part of the plate beneath. Of course, the wire and the colony cannot be simultaneously looked upon. When the colony is distinctly seen the platinum wire appears as a shadow, but the endeavor should be to make the end of the shadow which corresponds to the point of the wire appear exactly over the colony. It is then gradually depressed until it touches the colony and can be seen to break up and remove some of its substance; or should the colony be tough and coherent, to tear it away from the culture medium. It requires almost as much skill to withdraw the wire from the colony without touching anything as to successfully approach the colony in the first place. The bacterial mass adhering to the wire is now spread upon the surface of agar-agar or stabbed in gelatin or stirred in fluid medium, as the case may be. The higher the magnification under which this operation is done, the more difficult it is. Therefore only low-power lenses should be employed. The transplantation should be made immediately after the wire has touched the colony, the wire not being permitted in the meantime to come into contact with any other object. Immediately after receiving the inoculation, the tube containing the culture medium, held in the left hand, should be passed with a twisting motion through the flame of a Bunsen burner, so that its mouth and the cotton plug shall be heated. The cotton plug of course takes fire, but by vigorously blowing once or twice the fire can be extinguished. The cotton is then removed with a twisting motion of the plug and placed between two fingers of the left hand in such a manner that only the extra-tubular portion of the cotton is touched by the fingers. During the time that the plug is being withdrawn the tube should be held as nearly as possible in a horizontal position, in order that nothing may fall into it from the atmosphere. The inoculation having been made, the plug is returned and the platinum wire heated to redness in order that any remaining bacteria may be killed by the heat.

The Gelatin Puncture Culture.—To make satisfactory puncture cultures, the gelatin must be firm and not old and dry. Should the gelatin be soft and semi-fluid at the time the puncture is made, the bacteria diffuse themselves throughout and the typical appearance of the growth may be masked. On the other hand, if the gelatin is old, dry, or retracted, it is very apt to crack after the culture has been

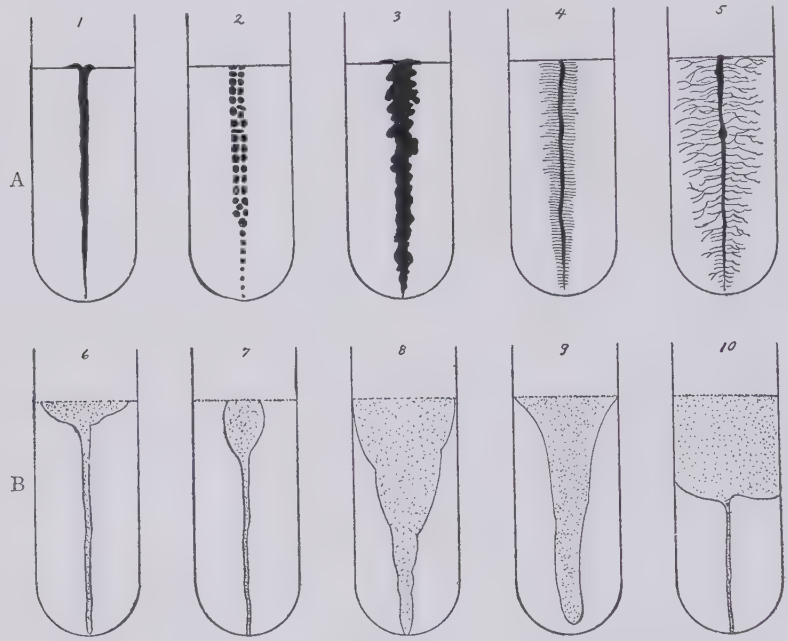


Fig. 48.—Types of growth in stab cultures. A, Non-liquefying: 1, Filiform (B. coli); 2, beaded (Str. pyogenes); 3, echinate (Bact. acidi-lactici); 4, villous (Bact. murisepticum); 5, arborescent (B. mycoides). B, Liquefying: 6, Crateriform (B. vulgare, 24 hours); 7, napiform (B. subtilis, 48 hours); 8, infundibuliform (B. prodigiosus); 9, saccate, (Msp. Finkleri); 10, stratiform (Ps. fluorescens) (Frost)

made and thus entirely destroy the characteristics of the growth. The wire used in the operation should be perfectly straight, and the puncture should be made from the center of the surface directly down to the bottom of the tube and then withdrawn, so that a simple puncture is made. The resulting appearances as the growth progresses are subject to striking variations according to the liquefying or non-

liquefying tendency of the micro-organisms. Various types of gelatin cultures are shown in the accompanying diagrams, and it is rather important that the student should familiarize himself with the terms by which these different growths are described, in order that uniformity of description may be maintained. Gelatin cultures may not be kept in the incubator oven, as the medium liquefies at such temperatures. On the other hand, it must not be kept where the temperature is too low, else the bacterial growth may be retarded. The temperature of a comfortably heated room, not subject to excessive variations, such as are caused by steam heat and the burning of gas, etc., is about the most appropriate. Like the colonies, the cultures must be carefully examined from day to day, as it not infrequently happens that a growth

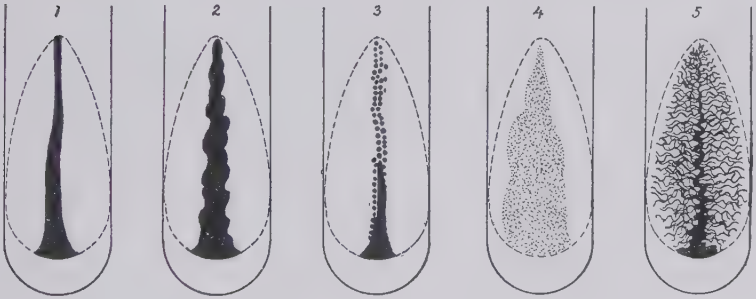


Fig. 49 —Types of streak cultures: 1, Filiform (B. coli); 2, echinulate (Bact. acidi-lactici); 3, beaded (Str. pyogenes); 4, effuse (B. vulgaris); 5, arborescent (B. mycoides) (Frost).

which shows no signs of liquefaction to-day may begin to liquefy to-morrow or a week hence, or even as late as two weeks hence.

The Agar-agar Culture.—Different operations vary in the exact method adopted in transplanting to agar-agar. In most cases, a simple stroke is made from the bottom of the tube in which the agar-agar has been obliquely solidified, and where it is fresh and moist, to the upper part, where it is thin and dry. In addition to this, it is advisable to make a puncture from the center of the oblique surface to the bottom of the tube. This enables us to tell whether the bacteria can grow as readily below the surface as above. Some workers always make a zigzag stroke upon the surface of the agar-agar. This does not seem to have any particular

advantage except in cases where it is desired to scatter the transplanted organisms as much as possible, in order that the bacteria will grow, or in order that a large bacterial mass may be secured.

Cultures upon Potato.—These are made by simply stroking the surface of the culture medium, the opacity of the potato making it impracticable to puncture it.

Cultures in Fluid Media.—Here, as has already been stated, transplantation consists in simply stirring in the bacteria so as to distribute them fairly well throughout the medium.

Adhesion Preparations.—Sometimes it is desirable to preserve an entire colony as a permanent microscopic specimen. To do this a perfectly clean cover-glass, not too large in size, is momentarily warmed, then carefully laid upon the surface of the gelatin or agar-agar containing the colonies. Sufficient pressure is applied to the surface of the glass to exclude bubbles, but not to destroy the integrity of the colony. The cover is gently raised by one edge, and if successful the whole colony or a number of colonies, as the case may be, will be found adhering to it. It is treated exactly as any other cover-glass preparation—dried, fixed, stained, mounted, and kept as a permanent specimen. It is called an *adhesion preparation*—"*Klatschpräparat.*"

Special Methods of Securing Pure Cultures.—Pure cultures from single colonies may also be secured by a very simple manipulation suggested by Banti.* The inoculation is made into the water of condensation at the bottom of an agar-agar tube, without touching the surface. The tube is then inclined so that the water flows over the agar, after which it is stood away in the vertical position. Colonies will grow where bacteria have been floated upon the agar-agar, and may be picked up later in the same manner as from a plate.

When the bacterium to be isolated (gonococcus, etc.) will not grow upon the media capable of alternate solidification and liquefaction, the blood-serum, potato, or other medium may be repeatedly stroked with the platinum wire dipped in the material to be investigated. Where the first strokes were made, confluent impure cultures occur; but as the wire became freer of organisms by repeated contact with the medium, the colonies become scattered and can be studied and transplanted.

*"Centralbl. f. Bakt. u. Parasitenk.," 1895, XVII, No. 16.

In some cases pure cultures may be most satisfactorily secured by animal inoculation. For example, when the tubercle bacillus is to be isolated from milk or urine which contains bacteria that would outgrow the slow-developing tubercle bacillus, it is necessary to inject the fluid into the abdominal cavity of a guinea-pig, await the development of tuberculosis in the animal, and then seek to secure pure cultures of the bacillus from the unmixed infectious material in the softened lymphatic glands.

In many cases, when it is desired to isolate Micrococcus tetragenus, the pneumococcus, and others, it is easier to inoculate the animal most susceptible to the infection and recover it from the blood or organs, than to plate it out and search for the colony among many others similar to it.

The growth upon agar-agar is in many ways less characteristic than in gelatin, but as the medium does not liquefy except at a high temperature (100° C.), it has that advantage. The colorless or almost colorless condition of the preparation also aids in the detection of chromogenesis.

Sometimes the growth is colored; at times the production of soluble pigment colors the agar-agar as well as the growth; sometimes the bacterial mass has one color and the agar-agar another. The growth may be filamentous, or simply a smooth, shining band. Occasionally the bacterium does not grow upon agar-agar unless glycerin be added (tubercle bacillus); sometimes it will not grow even then (gonococcus).

Still less characteristic are the growths upon potato. Most bacteria produce smooth, shining, irregularly extending growths, that may show characteristic colors.

In milk and litmus milk one should observe change in color from the occurrence of acid or alkali production, coagulation, gelatinization, and digestion of the coagulum.

Blood-serum is liquefied by some bacteria, but the majority of organisms have no characteristic reaction upon it. A few, as the bacillus of diphtheria, are, however, characterized by rapid development at given temperatures.

While most of the saprophytic bacteria grow well at the temperature of a well-warmed room, the important pathogenic forms must be kept at the temperature of the body either to permit growth or to secure typical development. To do this satisfactorily an incubating oven or thermostat becomes a necessity. Various forms, of wood and metal, are in the market, one being shown in the illustration (Fig. 50).

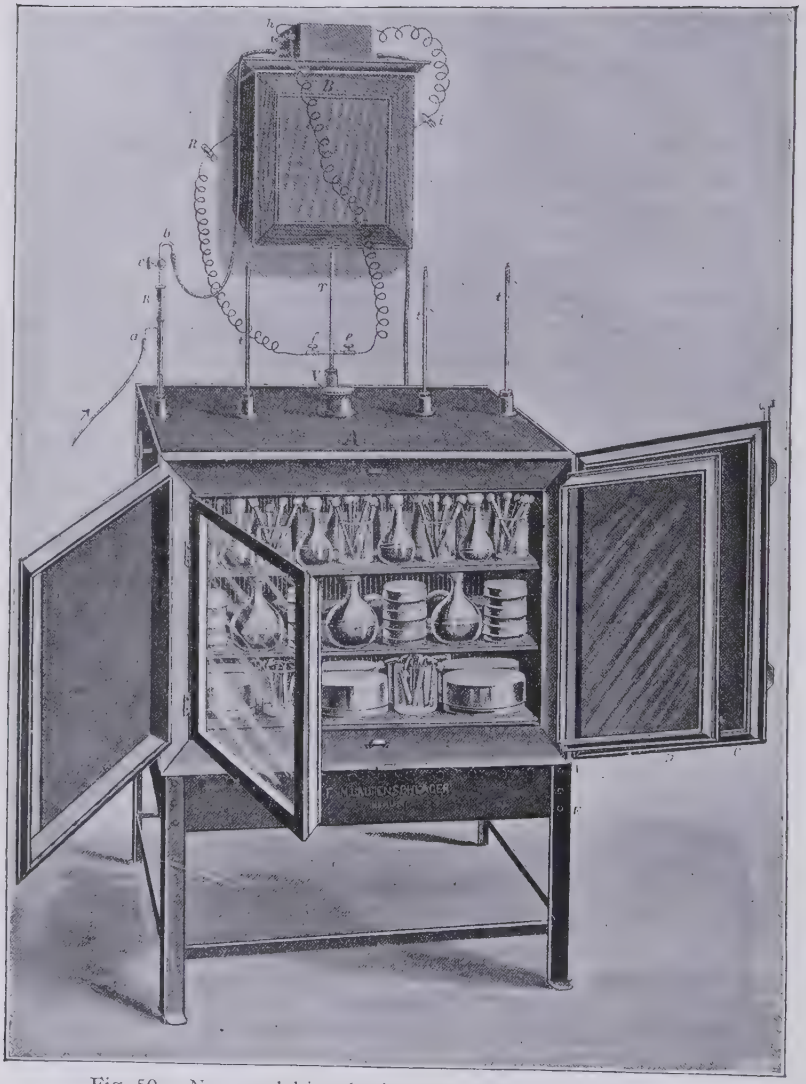


Fig. 50.—New model incubating oven with electro-regulator.

The growth in gelatin is generally so far removed from the walls of the tube (a central puncture nearly always being made in the culture medium, in order that the growth be symmetric) that it is impossible to make a microscopic ex-

amination of it with any power beyond that given by a hand-lens.

MICROSCOPIC STUDY OF CULTURES.

Some attention has been given to the preparation of microtome sections of the gelatin growth, which can be done if the glass be warmed just sufficiently to permit the gelatin containing the growth to be removed and placed in Müller's fluid (bichromate of potassium 2–2.5, sulphate of sodium 1, water 100), where it is hardened. When quite firm it is washed in water, passed through alcohols ascending in strength from 50 to 100 per cent., embedded in celloidin, cut wet, and stained like a section of tissue.

A ready method of doing this has been suggested by Winkler,* who bores a hole in a block of paraffin with the smallest size cork-borer, soaks the block in bichlorid solution for an hour, pours liquid gelatin into the cavity, allows it to solidify, inoculates it by the customary puncture of the platinum wire, allows it to develop sufficiently, and when ready cuts the sections under alcohol, subsequently staining them with much diluted carbol-fuchsin.

Neat museum specimens of plate and puncture cultures in gelatin can be made by simultaneously killing the microorganisms and permanently fixing the gelatin with formaldehyd, which can either be sprayed upon the gelatin or applied in dilute solution. As gelatin fixed in formaldehyd cannot subsequently be liquefied, such preparations will last indefinitely.

Standardizing Freshly Isolated Cultures.—This is a matter of some importance, as in bringing bacteria into the new environment of artificial cultivation their biologic peculiarities are temporarily altered, and it takes some time for them to recover themselves. While the appearances of the freshly isolated organism should be carefully noted, too much stress should not be laid upon them, and before beginning the systematic study of any new organism it should be made to grow for several successive generations upon two or three of the most important culture media. Its saprophytic existence being thus established, the characteristics manifested become the permanent peculiarities of the species.

*"Fortschritte der Medicin," Bd. xi, 1893, No. 22.

CHAPTER IX.

THE CULTIVATION OF ANAEROBIC BACTERIA.

The presence of uncombined oxygen in ordinary cultures inhibits the development of anaerobic bacteria. When such are to be cultivated, it therefore becomes necessary to utilize special apparatus or adopt physical or chemic methods for the exclusion of the air. Many methods have been suggested for the purpose, an excellent review of which has recently been published by Hunziker,* who divides them as follows, according to the principle by which the anaerobiosis is brought about:

1. By the formation of a vacuum.
2. By the displacement of the air by inert gases.
3. By the absorption of the oxygen.
4. By the reduction of the oxygen.
5. By the exclusion of atmospheric air by means of various physical principles and mechanical devices.
6. By the combined application of any two or more of the above principles.

This classification makes such an excellent foundation for the description of the methods that it has been unhesitatingly adopted.

1. Withdrawal of the Air and the Formation of a Vacuum.—This method was first suggested by Pasteur and was later modified by Roux, Gruber, Zupinski, Novy, and others. It is now very rarely employed. The appropriate container, whether a tube, flask, or some special device such as the Novy jar (Fig. 51), receives the culture, and then has the air removed by a vacuum pump, the tube either being sealed in a flame or closed by a stop-cock to prevent the reentrance of the air

2. Displacement of the Air by Inert Gases.—This method is decidedly preferable to the preceding, as it

* "Journal of Applied Microscopy and Laboratory Methods," March, April, and May, 1902; vol. v, Nos. 3, 4, and 5.

leaves no vacuum. It is easier to displace the oxygen than to withdraw it, and any apparatus permitting a combination of both features, as that designed by Ravenel,* from which the air can be sucked by a pump, to be later replaced by hydrogen, can be viewed with favor.

The most simple apparatus of the kind was suggested by Fränkel (Fig. 52), who inoculated a culture-tube of melted gelatin or agar-agar, solidified it upon the wall of the tube, as suggested by Esmarch, substituted for the cotton stopper a sterile rubber cork containing a long entrance and short exit tube of glass, passed hydrogen through the tube until the oxygen had been entirely removed, then sealed the ends in a flame. In this tube the growth of superficial and deep colonies can be observed. Hansen and Liborius con-

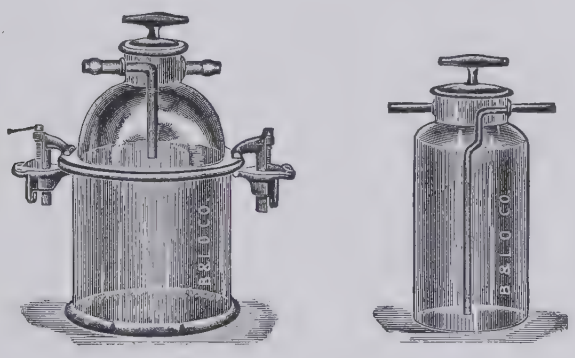


Fig. 51.—Novy's jars for anaerobic cultures.

structed special tubes (Fig. 53) by fusing a small glass tube into the wall of a culture-tube, and narrowing the upper part of the tube in a flame. After inoculation, hydrogen is passed into the small tube and permitted to escape through the mouth of the large tube until the air is entirely replaced, after which both tubes are sealed in a flame.

Instead of having a special apparatus for each culture, it is far better to adapt the principle to some larger piece of apparatus that can contain a number of tubes or Petri dishes at a time. For this purpose the jar invented by Novy or the apparatus of Botkin can be used.

The Novy jar receives as many inoculated tubes as it will

* "Bacteria of the Soil," "Memoirs of the National Academy of Sciences," First Memoir, 1896.

contain and has its stopper so replaced that the openings in the neck and stopper correspond. Hydrogen gas is passed through until the air is displaced. This usually takes several hours, as the cotton stoppers retain the air in the test-tubes and prevent rapid diffusion. When the air is all displaced, the stopper is turned so that the tubes are closed. If it be desired to expedite matters a pump can be used to withdraw the air, after which the hydrogen is permitted to enter.

Botkin's apparatus is intended for cultures in Petri dishes.

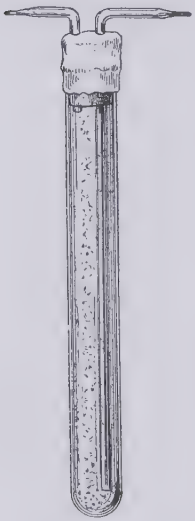


Fig. 52.—Fränkel's method of making anaerobic cultures.

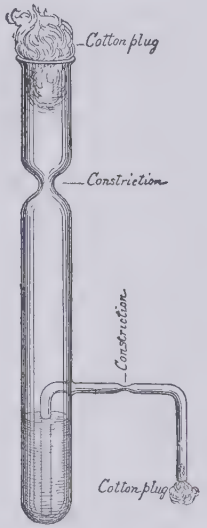


Fig. 53.—Liborius' tube for anaerobic cultures.

It consists of three parts—a deep dish of glass (*b*), a stand to support the Petri dishes to be exposed (*c*), and a bell-glass (*a*) to cover the stand and fit inside of the dish. It can easily be understood by reference to figure 54. The prepared dishes are stood uncovered in the rack, which is then placed in the dish forming the bottom of the apparatus, and into which liquid paraffin is poured to a depth of about two inches. The bell-glass cover is now stood in place and hydrogen gas is conducted through previously arranged rubber tubes (*d*, *e*). As soon as the air is displaced

through tube *d*, both tubes are withdrawn. It is well to place one Petri dish containing alkaline pyrogallic acid in the rack to absorb any oxygen not successfully displaced.

3. The Absorption of the Atmospheric Oxygen.—This method was first suggested by Buchner, whose idea was to absorb the atmospheric oxygen by alkaline pyrogallic acid and permit the bacteria to develop in the indifferent nitrogen. Various methods have been suggested for achieving this end, Buchner's own method consisting in the use of two tubes, a small one to contain the culture (Fig. 55) and a larger one to contain the absorbing fluid. A fresh solution of pyrogallic acid and sodium hydroxid were poured into the large tube, the smaller tube placed within it, upon some appropriate support, and the whole tightly corked.

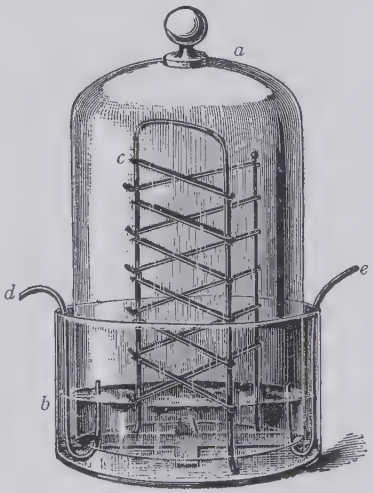


Fig. 54.—Botkin's apparatus for making anaerobic cultures.

Wright has given the most simple modification by suggesting that the cotton stopper of the ordinary culture-tube have its projecting part cut off and the plug itself pushed down the tube for a short distance. Some alkaline pyrogallic acid solution is poured upon the cotton, to saturate it, and the tube tightly corked.

4. Reduction of Oxygen.—Pasteur and, later, Roux have recommended the cultivation of anaerobic bacteria in association with aerobic bacteria by which the oxygen was to be absorbed. This method is too crude to be employed at the present time, as it destroys the essential characteristics of the cultures by mixing the products of the bacteria.

Chemic reduction of the oxygen has been attempted by the addition of 2 per cent. of glucose, as suggested by Liborius, 0.3–0.5 per cent. of sodium formate, as suggested by Kitasato and Weil, 0.1 per cent. of sodium sulphate, suggested by the same authors, and various other chemicals. None of these additions has been sufficiently successful to

merit continued favor, and at the present time this method is not employed.

5. Exclusion of Atmospheric Oxygen by Means of Various Physical Principles and Mechanical Devices.—This has appealed to the ingenuity of many experimenters, and many means of accomplishing the atmospheric exclusion have been tried with success.

The most simple plan is that of Hesse, who made a deep puncture in recently boiled and rapidly cooled gelatin or agar-agar, then covered the surface of the medium with sterile oil (Fig. 56). The so-called "shake culture" is an-

Fig. 55.—Buchner's method of making anaerobic cultures.

Fig. 56.—Hesse's method of making anaerobic cultures.

other very simple method, suggested by Liborius and Hesse. The medium to be inoculated, contained in a well-filled tube or flask, is boiled to displace the contained air, cooled so as no longer to endanger the introduced bacteria, then inoculated, the inoculated bacteria being distributed by gently shaking. On cooling, the medium "sets," the organisms below the surface remaining under anaerobic conditions.

Kitasato first used paraffin as a covering for the inoculated medium, his recommendation having recently been revived and made successful use of by Park for the cultivation of the tetanus bacillus. The paraffin floats upon the surface of the

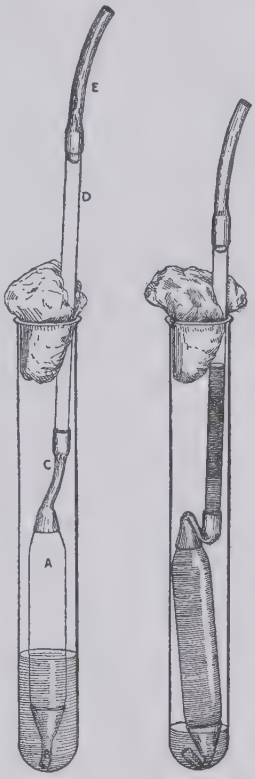
medium, melts during sterilization, but does not mix with it, and "sets" when cool. The inoculation is to be made while the culture medium is warm, after boiling and before the paraffin sets.

Koch studied the colonies of anaerobic organisms by cultivating them upon a film of gelatin covered by a thin sheet of sterilized mica, by which the air was excluded.

Salomonsen has made use of a pipet for making anaerobic cultures. It is made of a glass tube a few millimeters in diameter, drawn out to a point at each end. The inoculated gelatin or agar-agar is drawn in while liquefied and the ends sealed. The tube, of course, contains no air, and perfect anaerobiosis results.

In the chapter upon "Tetanus" I describe a method of cultivating anaerobic organisms that gave excellent results, though its application is limited, as it applies only to broth cultures and best to large quantities.

Theobald Smith has found the fermentation-tube and various modifications of it excellently well adapted to the growth of anaerobes, which, of course, grow only in the closed limb.



Figs. 57, 58.—Wright's method of making anaerobic cultures in fluid media (Mallory and Wright).

Hens' eggs have been used for anaerobic cultures, and in them the tetanus bacillus grows remarkably well. Conditions of anaerobiosis are, however, not perfect, as can be shown by the behavior of the egg itself. If oxygen be completely shut out by oiling or varnishing the shell, a fertile egg will not develop.

A quite satisfactory and simple device for routine work with anaerobic organisms has been invented by Wright*

*"Jour. Boston Soc. of Med. Sci.," Jan., 1900.

(Figs. 57 and 58). The essential feature consists of a pipet, D, with a rubber tube, E, at the end, and one interruption connected by a rubber tube, C. The device will be made clear at once by a glance at the accompanying illustration. The method of employment is very simple. An ordinary tube of bouillon or other fluid culture media receives the pipet, the whole being sterilized, the cotton plug in place. The bouillon being inoculated with the culture or secretion to be studied is drawn up in the bulb of the pipet, A, by suction, until it passes the rubber interruption, C. By forcing the upper end of the pipet downward in the test-tube, a kink is given each rubber tube and the fluid contained in the bulbous part of the pipet becomes hermetically sealed.

In all cases where the presence of suspected micro-organisms is to be demonstrated, it is necessary to make both aerobic and anaerobic cultures. For routine work of this kind, this method of Wright is probably the most convenient yet suggested.

CHAPTER X.

EXPERIMENTATION UPON ANIMALS.

The principal objects of medical bacteriology are to discover the cause, explain the symptoms, and bring about the cure and future prevention of disease. We cannot hope to achieve these objects without experimentation upon animals, in whose bodies the effects of bacteria and their products can be studied.

No one should more heartily condemn wanton cruelty to animals than the physician. Indeed, it is hard to imagine men, so much of whose life is spent in relieving pain, and who know so much about pain, being guilty of the butchery and torture accredited to them by a few of the laity, whose eyes, but not whose brains, have looked over the pages of physiologic text-books, and whose "philanthropy has thereby been transformed to zoolatry."

It is entirely through experimentation upon animals that we have attained our knowledge of physiology, most of our important knowledge of therapeutics, and most of our knowledge of the infectious diseases. Without its aid we would still be without one of the greatest achievements of medicine, the *serum therapy of diphtheria.*

Experiments upon animals, therefore, must be made, and, as the lower animals differ in their susceptibility to diseases, large numbers and different kinds of animals must be employed.

The bacteriologic methods are fortunately not cruel, the principal modes of introducing bacteria into the body being by subcutaneous, intraperitoneal, and intravenous injection.

Any hypodermic syringe that can conveniently be cleaned and disinfected may be employed for the purpose. Forms expressly designed for bacteriologic work and most frequently employed are shown in figure 59. Those of Meyer and Roux resemble ordinary hypodermic syringes; that of Koch is supposed to possess the decided advantage of not

having a piston to come into contact with the fluid to be injected. This is, however, really disadvantageous, inasmuch as the cushion of compressed air that drives out the contents is elastic, and unless carefully watched will follow the injection into the body of the animal. In making subcutaneous injections there is no disadvantage or danger from the entrance of air, but in intravenous injections it is extremely dangerous.

Syringes with metal or glass pistons are excellent, though not very durable. All syringes should be disinfected with 5 per cent. carbolic acid solutions *before* and *after* using, the carbolic acid being allowed to act for some time and then washed out with sterile water. Syringes should not often be

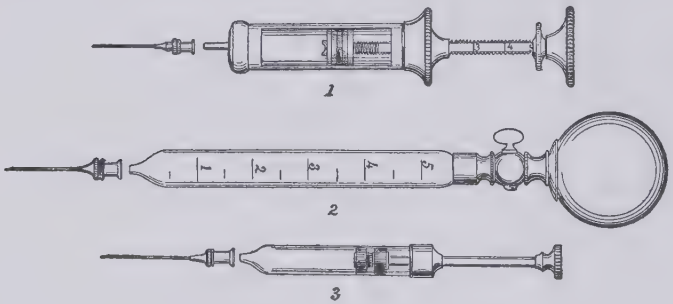


Fig. 59.—1, Roux's bacteriologic syringe; 2, Koch's syringe; 3, Meyer's bacteriologic syringe.

boiled, as it ruins the packings, whether of asbestos, leather, or rubber.

The intravenous injections differ only in that the needle of the syringe is introduced into a vein. This is easy to achieve in a large animal, like a horse, but is very difficult in a small animal, and well-nigh impossible in anything smaller than a rabbit. Such injections, when given to rabbits, are usually made into the ear-veins, which are most conspicuous and accessible (Fig. 60). A peculiar and important fact to remember is that the less conspicuous *posterior vein* of the ear is much better adapted to the purpose than the anterior. The introduction of the needle should be made from the *hairy external surface* of the ear when the vein is immediately beneath the skin.

If the ear be manipulated for a moment or two before the

injection, vasomotor dilatation occurs and the blood-vessels become larger and more conspicuous. The vein should be compressed at the root of the ear until the needle is introduced, and the injection made as near the root as possible. The fluid should be slowly injected.

Bacteria can be introduced into the lymphatics only by injecting liquid cultures of them into some organ with comparatively few blood-vessels and large numbers of lymphatics. The testicle is best adapted to this purpose, the needle being introduced deeply into the organ.

Sometimes subcutaneous inoculations are made by introducing the platinum wire through a small opening made in the skin by a snip of the scissors. By this means solid cultures from agar-agar, etc., can be introduced.

Fig. 60.—Method of making an intravenous injection into a rabbit. Observe that the needle enters the posterior vein from the hairy surface.

Intra-abdominal and intrapleural injections are sometimes made, and in cases where it becomes necessary to determine the presence or absence of the bacilli of tuberculosis or glanders in fragments of tissue it may be necessary to introduce small pieces of the suspected tissue under the skin. To do this the hair is closely cut over the point of election, which is generally on the abdomen, near the groin, the skin picked up with forceps, a snip made through it, and the points of the scissors introduced for an inch or so and then separated. By this manœuver a subcutaneous pocket is formed, into which the tissue is easily forced. The opening should not be large enough to require subsequent stitching.

When tissue fragments or collodion capsules are to be introduced into the abdominal cavity, the animal should be

anesthetized and a formal laparotomy done, the wound being carefully stitched together.

When, in studying Pfeiffer's phenomenon and similar conditions, it is desirable occasionally to withdraw drops of fluid from the abdominal cavity, a small opening can be burned through with a blunt needle. This does not heal readily, and through it, from time to time, a capillary pipet can be introduced and the fluid withdrawn.

Small animals, such as rabbits and guinea-pigs, can be held in the hand, as a rule. Guinea-pig and rabbit-holders of various forms can be obtained from dealers in laboratory

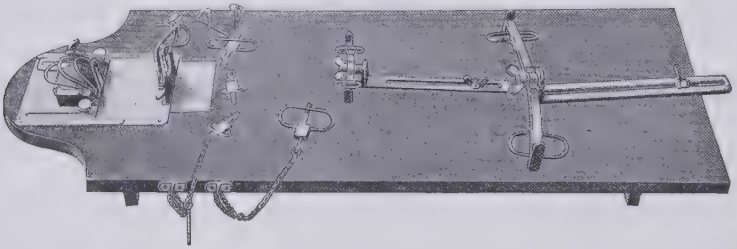


Fig. 61.—Latapie's animal holder for rabbits, guinea-pigs, and other small animals. This form of holder is in general use at the Institute Pasteur in Paris.

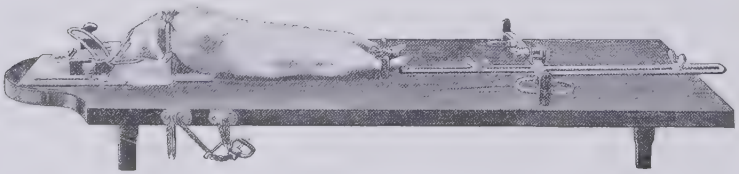


Fig. 62.—Guinea-pig confined in the holder.

supplies. The best of these is undoubtedly that of Latapie, shown in the accompanying illustration (Fig. 61). Dogs, cats, sheep, and goats can be tied and held in troughs. A convenient form of mouse-holder, invented by Kitasato, is shown in figure 63.

In all these experiments one must remember that the amount of material introduced into the animal must be in proportion to its size, and that injection experiments upon mice are usually so crude and destructive as to warrant the comparison drawn by Fränkel, that the injection of a few

minims of liquid into the pleural cavity of a mouse is "much the same as if one would inject through a fire-hose three or four quarts of some liquid into the respiratory organs of a man."

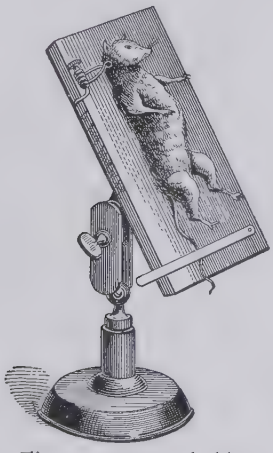


Fig. 63 —Mouse-holder.

When desired for experiments, the blood of animals can best be secured from the jugular vein. From small animals, such as rabbits and guinea-pigs, it may be secured by introducing a small cannula into the carotid artery, if the animal is to be kept alive, or by anesthetizing the animal, exposing the heart, cutting a small opening into a ventricle, and sucking up the blood with a pipet introduced through the opening. The blood of mice and rats can thus be secured.

Post-mortems.—Observation of experiment animals by no means ceases with their death. Indeed, he cannot be a bacteriologist who is not already a good pathologist and expert in the recognition of diseased organs.

When an autopsy is to be made upon a small animal, it is best to wash it for a few moments in a disinfecting solution, to kill the germs present upon the hair and skin, as well as to moisten the hair, which can then be much more easily kept out of the incision.

Small animals can be tacked to a board or tied, by cords fastened to the legs, to hooks soldered to the corners of an easily disinfected tray. The dissection should be made with

sterile instruments. When a culture is to be made from the interior of an organ, its surface should first be seared with a hot iron, a puncture made into it with a sterile knife, and the culture made by introducing a platinum wire.

If the bacteriologic examination cannot be made at once, the organs to be studied should be removed with aseptic precautions, wrapped in a sterile towel or a towel wet with a disinfecting solution, and carried to the laboratory, where the surface is seared and the necessary incisions made with sterile instruments.

Fragments intended for subsequent microscopic examination should be cut into small cubes (of 1 c.c.) and fixed in Zenker's fluid or absolute alcohol. (See page 141.)

Collodion capsules are quite frequently employed for the purpose of cultivating bacteria in a confined position in the body of an animal, where they can freely receive and utilize the body-juices without being subjected to the action of the phagocytes. In such capsules the bacteria usually grow plentifully, and not rarely have their virulence increased.

The capsules can be made of any size, though they are probably most easily handled when of about 5–10 c.c. capacity. The size is always an objection, because of the disturbance occasioned when they are introduced into the abdominal cavity.

The capsules are made by carefully coating the outside of the lower part of a test-tube with collodion until a sufficiently thick, homogeneous layer is formed. During the coating process the tube must be twirled alternately within and without the collodion, so that it is equally distributed upon its surface. When the desired thickness is attained, and the collodion is sufficiently firm, the tube is plunged under water and the hardening process checked.

A cut is next made around the upper edge of the collodion film, and it is removed by carefully turning it inside out. In this manner an exact mold of the tube is formed. If a small opening be made at the end of the tube over which the sac is molded, and the tube filled with water after being properly coated with collodion, a small amount of pressure, applied by blowing gently into the tube, will force the water between the collodion and glass and so detach it without inversion. A test-tube of the same size is next constricted to a degree that will not interfere with the future introduction of culture media in a fine pipet or inoculation with a platinum loop,

and that will permit of ready sealing in a flame when necessary; the rounded end is cut off, and the edges are smoothed in a flame. The upper open end of the collodion bag is carefully fitted over the end of the tube, shrunk on by a gentle heating, and cemented fast with a little fresh collodion applied to the line of union. Novy recommends that a thread of silk be wound around the point of union, to hold the collodion in place and to aid in handling the finished sac. It now appears as in figure 64, *b*. The sac is next filled with distilled water up to the thread, the tube is plugged with cotton, and the whole placed in a larger test-tube containing distilled water, the cotton plug being packed tightly around the smaller tube, so that the collodion sac does not reach the bottom of the large tube, but hangs suspended in the water it contains. The whole is now carefully sterilized by steam.

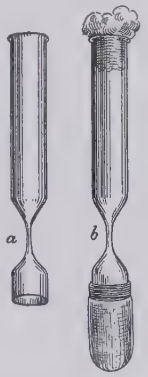


Fig. 64.—Preparation of collodion sacs: *a*, Test-tube constricted and cut; *b*, sac attached to the tube.

When ready for use, a tube of bouillon is inoculated with the culture intended to be placed in the animal, the water in the capsule is pipetted out and replaced by the inoculated bouillon carefully introduced with a pipet, the constricted portion is sealed in a flame, and the capsule picked up with forceps and introduced into the peritoneum by an aseptic operation.

The collodion capsules may be made of any size. Those for rabbit experiments should be of about 10 c.c. capacity, those for guinea-pig experiments about 5 c.c. By coating large glass tubes they can be made of 500 c.c. capacity, the large bags being useful for chemic dialysis.

CHAPTER XI.

THE DIFFERENTIATION OF BACTERIA.

The most difficult thing in bacteriology is the determination of the species of bacteria that come under observation.

A few micro-organisms are characteristic in morphology and in their chemic and other products, and present no difficulty. Thus, the tubercle bacillus is characteristic in its reaction to the anilin dyes, and can usually be recognized by this peculiarity. Some, as Bacillus mycoides, have characteristic agar-agar growths. The red color of Bacillus prodigiosus and the blue of Bacillus janthinus speak almost positively for them. The potato cultures of Bacillus mesentericus fuscus and vulgatus are usually sufficient to enable us to recognize them. Unfortunately, however, there are several hundreds of described species that lack any one distinct characteristic that may be used for differential purposes, and require that for their recognition we shall wellnigh exhaust the bacteriologic technic in order to determine them.

Useful tables have been compiled by Eisenberg, Migula, Chester, and others, and are indispensable to the worker. The most useful is probably the "Manual of Determinative Bacteriology," by F. D. Chester (1901), from which, through the courtesy of the author and publisher, the following synopsis of groups is taken. Unfortunately, in tabulating bacteria we constantly meet species described so insufficiently as to make it impossible to properly classify and tabulate them.

The only way to determine a species is to study it thoroughly, step by step, and compare it with the descriptions and tables. In this regard the differentiation of bacteria resembles the determination of the higher plants with the aid of a botanic key, or the qualitative analysis for the detection of unknown chemic compounds. Such a key for specific bacterial differentiation is really indispensable even though it be imperfect, and every student engaged in research

STANDARD CHART FOR BACTERIAL ANALYSIS.

NAME ..... SOURCE ..... HABITAT..... ..... DATE..... REPORTED BY .....

Form and arrangement in bouillon, grown ..... hours at 18°–20° C.; ditto, grown ..... hours at 36°–38° C.

Micrococcus, single, pairs, chains, tetrads, or cubical packets; *Bacillus*, single, pairs, chains, or filaments; *Spirillum*, comma, spiral.

Size, length ..... μ, breadth ..... μ; extreme lengths from ..... μ to ..... μ.

Capsules, none observed, easily observed or demonstrated. Conditions under which they are present, agar, serum, milk, or .....

Spores, none observed within ..... hours at .....° C. on .... When present are polar, central, cells ..... swollen.

Germinate within ..... hours at .....° C. Stain by ..... method. Are killed at 100° C. in .... minutes.

Vacuoles, observed when grown on ..... at .....° C., or when stained with .....

Motility, sluggish or active, rotary or direct, more pronounced in ..... cultures grown at ..... ° C. for .. .... hours.

Flagella stain by .... method; are monotrichous, lophotrichous, amphitrichous, peritrichous.

Pleomorphism, observed in .... cultures grown at .....° C. for ..... days.

Stain, easily or with difficulty with ....., uniformly or irregularly. Stained or decolorized by Gram's method.

GELATIN OR AGAR PLATES.	Surface colonies.		Deep colonies.	
	Gelatin.	Agar.	Gelatin.	Agar.
Size.				
Shape.				
Margin.				
Textures.				
Color.				
Under mica plate.				

GELATIN OR AGAR-TUBE.

Puncture.

Form.
Surface growth.
Size.
Shape.
Margin.
Texture.
Color.

BOUILLON.

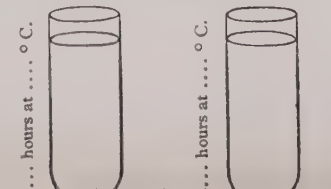
Opacity begins after .... hrs. at ....° C.
Pellicle forms in .... hrs. at ....° C.
Color .... appears in ... hrs. at ....° C.
Thickness.
Consistence.
Deposit forms in .... hrs. at ....° C.
Amount.
Color.
Character, compact, flocculent, granular, flaky, or viscid on agitation.
Color.
Odor.
Reaction, ... after ....hrs. at ....° C.

POTATO OR BLOOD-SERUM.

Size.
Shape.

SKETCH OF GERM AND COLONY.

STAB CULTURES.



Shape.
Margin.
Surface relief.
Light transmission.
Color.
Luster.
Consistence.

Change in medium.

Color.
Consistence.
Odor.

Liquefaction.
Gas production.

NITRATE BROTH.

Reduction of nitrates.
Dextrose-free bouillon.
Indol production.

.... hours at .... °

.... hours at .... °

MILK.

Curd, curdles or does not curdle in .... days at 18°–20° C. or 36°–38° C.
" " " " " on boiling after .... days at ....° C.
hard or soft, in one mass or in fragments, gas-bubbles.
changed or unchanged by boiling.
Whey, separates from curd or not, amount, transparent or turbid.
Reaction, ... in ..... days at ....° C. *Color.* *Gas production.*
Digestion becomes gradually transparent without forming curds.
complete in .... days at ....° C.
effects of boiling, odor, clear or cloudy, watery or viscid.
reaction at end of digestion .....

SUGAR BOUILLON IN FERMENTATION-TUBES	Amount of gas in % formed at 18°–20° C.						Amount of gas in % formed at 36°–38° C.					
	in 1 d.,	2 ds.,	3 ds.,	....	days.		in 1 d.,	2 ds.,	3 ds.,	....	days.	
Dextrose	....	....	....	....	....	....	....	....	....	....	....	....
Lactose	....	....	....	....	....	....	....	....	....	....	....	....
Saccharose	....	....	....	....	....	....	....	....	....	....	....	....

Reaction of fluid in..... after....days at ....° C.	CO_2 %	H %	Pellicle.	Opacity.	Color.
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....
.....	.....	.....	.....	.....	.....

PIGMENT,
developed in presence or absence of oxygen.
" in ..... cultures at ..... ° C. in ..... hours.
color ....., changed to ..... by acid or alkali.
soluble in ....., spectrum .....

PATHOGENESIS.

OPTIMUM TEMPERATURE ....° C. GROWTH LIMITS ....° C. to ....° C. THERMAL DEATH-POINT .... ° C., time of exposure .... minutes.

PRODUCTION OF ACIDS OR ALKALIES.
Carbohydrates absent or present.

RELATION TO FREE OXYGEN.
Obligatory aërobe.
Facultative anaërobe.
Obligatory anaërobe.

RELATION OF GROWTH TO ACIDITY OR ALKALINITY OF MEDIUM.
.... % acid to .... % alkaline.

work should have one. As Chester says, "probably nine-tenths of the forms of bacteria already described might as well be forgotten or given a respectful burial. This will then leave comparatively few well-defined species to form the nuclei of groups in one or another of which we shall be able to place all new and sufficiently described forms." "That typical forms or species of bacteria do exist, no one can deny. These typical forms furthermore present certain definite morphologic, biologic, cultural, and perhaps pathogenic characters which establish the types independently of minor variations.

"The most marked of these types we select to become the centers of groups, around which are gathered all related species or varieties." "The division of the bacteria into groups, so far as grouping was possible, is outlined in the following tables:"

A PROPOSED SYNOPSIS OF GROUPS OF BACTERIA.

Bacterium.

I. Without endospores.
  A. Aerobic and facultative anaerobic.
    a. Gelatin not liquefied.
      * Decolorized by Gram's method.
        † Obligate aerobic. Acetic Ferment Group.
        †† Aerobic and facultative anaerobic.
          Gas generated in glucose bouillon.
            Gas generated in lactose bouillon. Bact. aerogenes Group.
            Little or no gas generated in lactose bouillon. Friedländer Group.
          No gas generated in glucose bouillon.
            Milk coagulated. Fowl Cholera Group.
            Milk not coagulated. Swine Plague Group.
      ** Stained by Gram's method.
        † Gas generated in glucose bouillon. Lactic Ferment Group.
    b. Gelatin liquefied.
      * Colonies on gelatin ameboid or proteus-like. Bact. radiatum Group.
      ** Colonies on gelatin round, not ameboid. Bact. ambiguum Group.
II. Produce endospores.
  1. No growth at room temperature, or below 22°–25° C. Thermophilic Group.
  2. Grow at room temperatures.
    a. Gelatin liquefied. Anthrax Group.
    b. Gelatin not liquefied. Bact. fæcalis Group.

Bacillus.

I. Without endospores.
  A. Aerobic and facultative anaerobic.

a. Gelatin colonies roundish, not distinctly ameboid.
* Gelatin not liquefied.
† Decolorized by Gram's method.
Gas generated in glucose bouillon.
Milk coagulated. COLON GROUP.
Milk not coagulated. HOG CHOLERA GROUP.
No gas generated in glucose bouillon. TYPHOID GROUP.
†† Stained by Gram's method. B. MURIPESTIFER GROUP.
** Gelatin liquefied.
† Gas generated in glucose bouillon. B. CLOACÆ GROUP.
†† No gas generated in glucose bouillon. Include a large number of bacteria not sufficiently described to arrange in groups.
b. Gelatin colonies ameboid, cochleate, or otherwise irregular.
* Gelatin liquefied. PROTEUS VULGARIS GROUP.
** Gelatin not liquefied. B. ZOPFI GROUP.

II. Produce endospores.
A. Aerobic and facultative anaerobic.
1. Rods not swollen at sporulation.
a. Gelatin liquefied.
* Liquefaction of the gelatin takes place slowly. Ferment urea, with strong production of ammonia. URO-BACILLUS GROUP OF MIQUEL.
** Gelatin liquefied rather quickly.
† Potato cultures rugose. POTATO BACILLUS GROUP.
†† Potato cultures not distinctly rugose. B. SUBTILIS GROUP.
b. Gelatin not liquefied. B. SOLI GROUP.
2. Rods spindle-shaped at sporulation. B. LICHENIFORMIS GROUP.
3. Rods clavate at sporulation. B. SUBLANATUS GROUP.
B. Obligate anaerobic.
1. Rods not swollen at sporulation. MALIGNANT EDEMA GROUP.
2. Rods spindle-shaped at sporulation. CLOSTRIDIUM GROUP.
3. Rods clavate-capitate at sporulation. TETANUS GROUP.

PSEUDOMONAS (Migula).

I. Cells colorless, without a red-colored plasma and without sulphur granules.
A. Grow in ordinary culture media.
1. Without endospores.
a. Aerobic and facultative anaerobic.
* Without pigment.
† Gelatin not liquefied.
Gas generated in glucose bouillon. PS. MONADIFORMIS GROUP.
No gas generated in glucose bouillon. PS. AMBIGUA GROUP.
†† Gelatin liquefied.
Gas generated in glucose bouillon. PS. COADUNATA GROUP.
No gas generated in glucose bouillon. PS. FAIRMONTENSIS GROUP.
* Produce pigment on gelatin or agar.
† Pigment yellowish.

Gelatin liquefied. Ps. OCHRACEA GROUP.
Gelatin not liquefied. Ps. TURCOSA GROUP.
†† Pigment blue-violet.
Gelatin liquefied. Ps. JANTHINA GROUP.
Gelatin not liquefied. Ps. BEROLINENSIS GROUP.
** Produce a greenish-bluish fluorescence in culture media.
† Gelatin liquefied. Ps. PYOCYANEA GROUP.
†† Gelatin not liquefied. Ps. SYNCYANEA GROUP.
2. With endospores, aerobic and facultative anaerobic.
a. Non-chromogenic.
* Rods not swollen at sporulation. Ps. ROSEA GROUP.
** Rods swollen at one end at sporulation. Ps. TROMMELSCHLÄGER GROUP.
b. Produce a greenish-bluish fluorescence in culture media.
* Gelatin liquefied. Ps. VIRIDESCENS GROUP.
** Gelatin not liquefied. Ps. UNDULATA GROUP.
B. Do not grow in nutrient gelatin or other organic media. NITRIMONAS GROUP.

II. Cell plasma with a reddish tint, also with sulphur granules. CHROMATIUM GROUP.

MICROSPIRA (Migula).

I. Cultures show a bluish-silvery phosphorescence. PHOSPHORESCENT GROUP.
II. Cultures not phosphorescent.
A. Gelatin liquefied.
1. Cultures show the nitro-indol reaction.
a. Very pathogenic to pigeons. MSP. METSCHNIKOVI GROUP.
b. Not distinctly pathogenic to pigeons. CHOLERA GROUP.
2. Nitro-indol reaction negative or very weak, at least after twenty-four hours. CHOLERA NOSTRAS GROUP.
B. Gelatin not liquefied or only slightly so. MSP. SAPROPHILA GROUP.

MYCOBACTERIUM (Lehmann-Neumann).

I. Stain with basic anilin dyes, and easily decolorized by mineral acids when stained with carbol-fuchsin.
A. Grow well on nutrient gelatin. Gelatin liquefied very slowly or merely softened.
1. Stain by Gram's method. SWINE ERYSIPELAS GROUP.
2. Not stained by Gram's method. GLANDERS GROUP.
B. Little or no growth in ordinary nutrient gelatin.
1. Grow well in nutrient bouillon at body temperatures.
a. Stained by Gram's method. Rods cuneate—clavate—irregularly swollen. DIPHTHERIA GROUP.
2. No growth in nutrient bouillon or on ordinary culture media. Rods slender, tubercle-like.
a. Stain by Gram's method. LEPROSY GROUP.
b. Do not stain by Gram's method. INFLUENZA GROUP.
3. No growth in nutrient bouillon or on ordinary culture media. Rods variable. ROOT-TUBERCLE GROUP.

II. Not stained with aqueous solutions of basic anilin dyes; not easily decolorized by acids. TUBERCLE GROUP.

COCCACEÆ.

Cells in their free condition globular, becoming slightly elongated before division. Cell division in one, two, or three directions of space.

A. Cells without flagella.
1. Division in only one direction of space. *Streptococcus* (Billroth).
2. Division in two directions of space. *Micrococcus* (Hallier).
3. Division in three directions of space. *Sarcina* (Goodsir).

B. Cells with flagella.
1. Division in two directions of space. *Planococcus* (Migula).
2. Division in three directions of space. *Planosarcina* (Migula).

CHAPTER XII.

THE BACTERIOLOGY OF THE AIR.

MICRO-ORGANISMS are almost universally suspended in the dust of the air, their presence being a constant source of contamination in our bacteriologic researches and occasionally a menace to our health.

Such aerial organisms are neither ubiquitous nor equally disseminated, but are much more numerous where the air is polluted and dusty than where it is pure. The purity of the atmosphere bears a distinct relation to the purity of the soil over which its currents blow.

The micro-organisms of the air are for the most part harmless saprophytes taken up and carried about by the wind. They are almost always taken up from dry materials, experiment having shown that they arise from the surfaces of liquids with much difficulty. Not all the micro-organisms of the air are bacteria, and a plate of sterile gelatin exposed to the air for a brief time will generally grow molds and yeasts as well.

In some cases the bacteria are pathogenic, especially where discharges from diseased animals have been allowed to collect and dry. On this account the atmosphere of hospital wards and of rooms in which infectious diseases are being treated is more apt to contain them than the air of the street. However, because of the expectoration from cases of tuberculosis, influenza, and pneumonia, which is often ejected upon the sidewalks and floors of public places, the presence of occasional pathogenic bacteria is far from uncommon in street-dust.

Günther points out that the greater number of the bacteria which occur in the air are cocci, sarcina being particularly abundant. Most of them are chromogenic and do not liquefy gelatin. It is unusual to find more than two or three varieties of bacteria at a time.

To determine whether bacteria are present in the air or not, all that is necessary is to expose a film of sterile gelatin

on a plate or Petri dish to the air for a while, cover, and observe whether or not bacteria grow upon it.

To make a quantitative estimation is, however, more difficult. Several methods have been suggested, of which the most important may be briefly mentioned:

Hesse's method is simple and good. It consists in making a measured quantity of the air to be examined pass through a horizontal sterile glass tube about 70 cm. long and 3.5 cm. wide (Fig. 65), the interior of which is coated with a film of gelatin in the same manner as an Esmarch tube. The

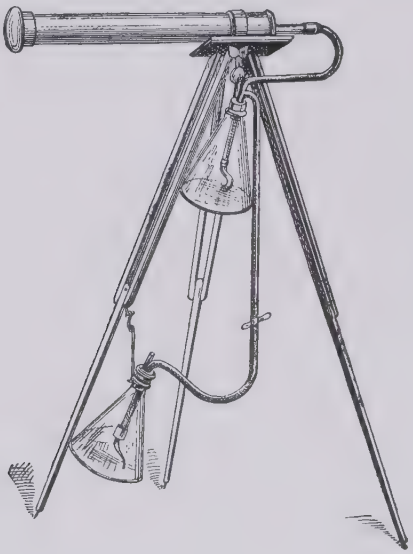


Fig. 65.—Hesse's apparatus for collecting bacteria from the air.

tube is closed at both ends with sterile corks carrying small glass tubes plugged with cotton. When ready for use the tube at one end is attached to a hand-pump, the cotton removed from the other end, and the air slowly passed through, the bacteria having time to sediment upon the gelatin as they pass. When the required amount has passed, the tubes are again plugged, the apparatus stood away for a time, and subsequently, when they have grown, the colonies are counted. The number of colonies in the tube will represent pretty accurately the number of bacteria in volume of air that passed through the tube.

In such a tube, if the air pass through with proper slowness, the colonies will be much more numerous near the point of entrance than near that of exit. The first to fall will probably be those of heaviest specific gravity—*i. e.*, the molds.

Petri's Method.—A more exact method is that of Petri, who uses small filters of sand held in place in a wide glass tube by small wire nets (Fig. 66). The sand used is made to pass through a sieve whose openings are of known size, is heated to incandescence, then arranged in the tube so that two of the little filters, held in place by their wire-gauze coverings, are superimposed. One or both ends of the tube are closed with corks having a narrow glass tube. The apparatus is sterilized by hot air, and is then ready for use. The method of employment is very simple. By means of a hand-pump 100 liters of air are made to pass through the filter in from ten to twenty minutes, the contained microorganisms being caught and retained by the sand. The sand

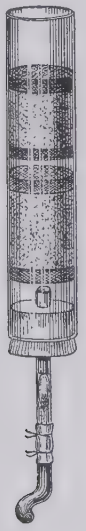


Fig. 66.—Petri's sand filter for air-examination.

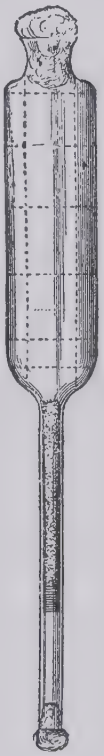


Fig. 67.—Sedgwick's expanded tube for air-examination.

from the upper filter is then carefully mixed with sterile melted gelatin and poured into sterile Petri dishes, where the colonies develop and can be counted. Petri points out in relation to his method that the filter catches a relatively greater number of bacteria in proportion to molds than the Hesse apparatus, which depends upon sedimentation. Sternberg points out that the chief objection to the method is the presence of the sand, which interferes with the recognition and counting of the colonies in the gelatin.

Sedgwick's Method.—Sedgwick and Miquel have recommended the use of a soluble material—granulated or pulverized sugar—instead of the sand. The apparatus used for the sugar experiments differs a little from the original of Petri, though the principle is the same, and can be modified to suit the experimenter.

A particularly useful form of apparatus (Fig. 67), suggested by Sedgwick and Tucker, has an expansion above the filter, so that as soon as the sugar is dissolved in the melted gelatin it can be rolled out into a film like that of an Esmarch tube. This cylindric expansion is divided into squares which make the counting of the colonies very easy.

Roughly, the number of germs in the atmosphere may be estimated at from 100 to 1000 per cubic meter.

The bacteriologic examination of air is of very little importance, because of the numerous errors that must be met. Thus, when the air of a room is quiescent it may contain very few bacteria; let some one walk across the floor so that dust rises, and the number of bacteria becomes considerably increased; if the room be swept, the increase is enormous. From these and similar contingencies it becomes very difficult to know just when and how the air is to be examined, and the value of the results is correspondingly lessened.

The most sensible studies of the air aim rather at the discovery of some definite organism or organisms than at the determination of the total number per cubic meter.

CHAPTER XIII.

BACTERIOLOGY OF WATER.

UNLESS water has been specially sterilized, received and kept in sterile vessels, it always contains some bacteria, the number usually bearing a distinct relationship to the quantity of organic matter present.

The majority of the water bacteria are bacilli, and are as a rule non-pathogenic. Wright,* in his examination of the bacteria of the water from the Schuylkill River, found two species of micrococci, two species of cladothrices, and forty-six species and two varieties of bacilli. Pathogenic bacteria, such as the spirillum of Asiatic cholera and the bacillus of

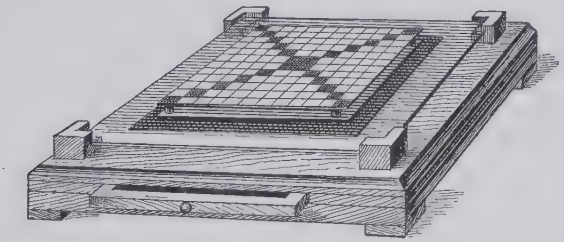


Fig. 68.—Wolfhügel's apparatus for counting colonies of bacteria upon plates.

typhoid fever, may occur in polluted water, but their occurrence is exceptional.

The method of determining the number of bacteria in water is very simple, and can be accomplished with very little apparatus. The method depends upon the equal distribution of a measured quantity of the water to be examined in some sterile liquefied medium, whose subsequent solidification in a thin layer permits the colonies to be counted.

The method originated with Koch, and may be performed with plates, Petri dishes, or Esmarch rolls. It is always best

* "Memoirs of the National Academy of Sciences," vol. VII, Third Memoir.

to make a number of cultures with different quantities of the water, using, for example, 0.01, 0.1, 0.5, and 1.0 c.c., respectively, to a tube of liquefied gelatin, agar-agar, or glycerin agar-agar.

The details of the method depend upon the quality of the water to be examined. If the number of bacteria per cubic centimeter be small, large quantities may be used; but if there be millions of bacteria in every cubic centimeter, it may be necessary to dilute the water to be examined in the proportion of 1 : 10 or 1 : 100 with sterile water, mixing well, and making the plate cultures from the dilutions.

It is best to count all the colonies developed upon the culture, if possible; but when hundreds or thousands are scattered over it, an estimate made by counting and averaging the number in each of the small squares of some counting apparatus, such as that devised by Wolfhügel (Fig. 68) or that of Esmarch (Fig. 69). In counting the colonies a lens is indispensable.

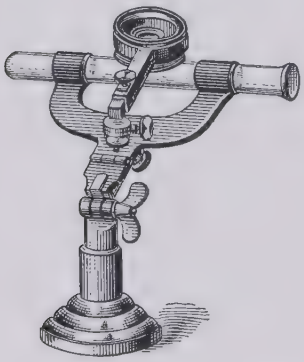


Fig. 69.—Esmarch's instrument for counting colonies of bacteria in Esmarch tubes.

The majority of the water bacteria rapidly liquefy gelatin, on which account it is better to employ both gelatin and agar-agar in making the cultures.

In ordinary city hydrant-water the bacteria number from 2 to 50 per cubic centimeter; in good pump-water, 100 to 500; in filtered water from rivers, according to Günther, 50 to 200; in unfiltered river-water, 6000 to 20,000. According to the pollution of the water the number may reach as many as 50,000,000.

The waters of wells and springs are dependent for their purity upon the character of the earth or rock through which they filter, and the waters of deep wells are much more pure than those of shallow wells, unless contamination take place from the surface of the ground.

Ice always contains bacteria if the water contained them before it was frozen. In Hudson River ice Prudden found an average of 398 colonies in a cubic centimeter.

A sample of water when collected for examination should

be placed in a clean sterile bottle or in a hermetically sealed pre-sterilized glass bulb, and must be examined as soon as possible, as the bacteria multiply rapidly in water which is allowed to stand for a short time. If the water to be examined must be transported any considerable distance before the manipulations are performed, it should be packed in ice. The greatest care must always be exercised that the unnatural conditions arising from the bottling of the water, the changes of temperature, and the altered relationship to light and the atmosphere, do not modify the number of contained bacteria.

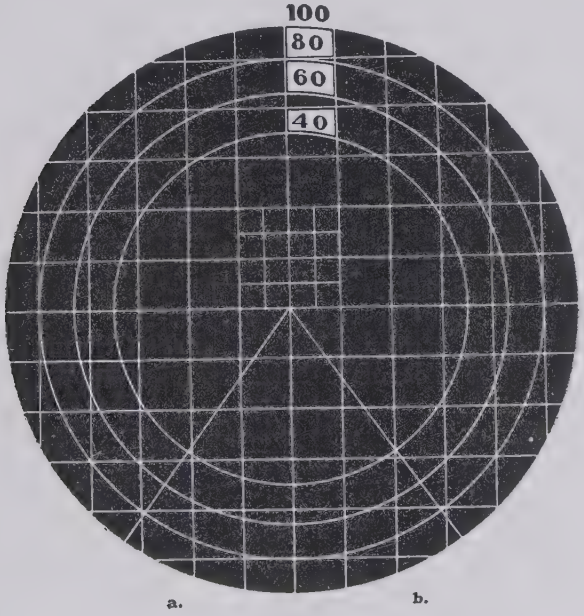


Fig. 70.—Frost's plate counter, for counting colonies of bacteria on Petri dish or plate cultures. The cross-lines divide the figure into square centimeters. The numbers at the top of the figure indicate the area in centimeters of the various discs. The area of each sector (a and b) is one-tenth of the whole area.

The detection of such important bacteria as the colon and typhoid bacilli, and the cholera spirillum, will be considered in the chapters treating of those respective organisms.

Unfortunately, the bacteriologic examination of waters

does not throw satisfactory light upon their exact hygienic usefulness. Of course, if cholera or typhoid fever bacteria are present, the water is dangerous, but the quality of the water cannot always be gauged by the number of bacteria it contains.

Drinking-water, especially that furnished to large cities, is not infrequently contaminated with sewage, and contains intestinal bacteria—Bacillus coli communis. For the ready determination of this organism, which is an important indication that the water is polluted, Smith * has made use of the fermentation-tube in addition to the plate. His method is to add to each of several fermentation-tubes containing 1 per cent. dextrose-bouillon a certain quantity of water. The evolution of 50–60 per cent. of gas by the third day is a strong indication that the colon bacillus is present. Plates may be used to confirm the presence of the bacillus, but are hardly necessary, as there is scarcely another bacterium met with in water that is capable of producing so much gas.

It was at one time thought that the occurrence of the colon bacillus in water was sufficient to condemn its potability, but the evidence accumulated in recent years, showing that this organism may reach streams from manured soil, may enter it with the dejecta of domestic animals, wild animals, birds, and perhaps even of fishes, makes it doubtful whether anything but an exceptionally large number of the organisms should be looked upon as indicative of sewage pollution and proof that the water is not potable.

In determining the species of bacteria found in the water reference must be made to the numerous monographs upon the subject and to special tables. An excellent table of this kind, arranged by Fuller,† is given on pages 236, 237.

Filtration with sand, etc., diminishes the number of bacteria for a time, but, as the organisms multiply in the filter, the benefit is not permanent and the filters must frequently be subjected to bacteriologic tests and the sand washed, spread out to dry and the filters renewed. Porcelain filters seem to be the only positive safeguard, and even these, the best of which seems to be the Pasteur-Chamberland, allow the bacteria to pass through if used too long without proper attention.

For those whose special line of work is the bacteriology of

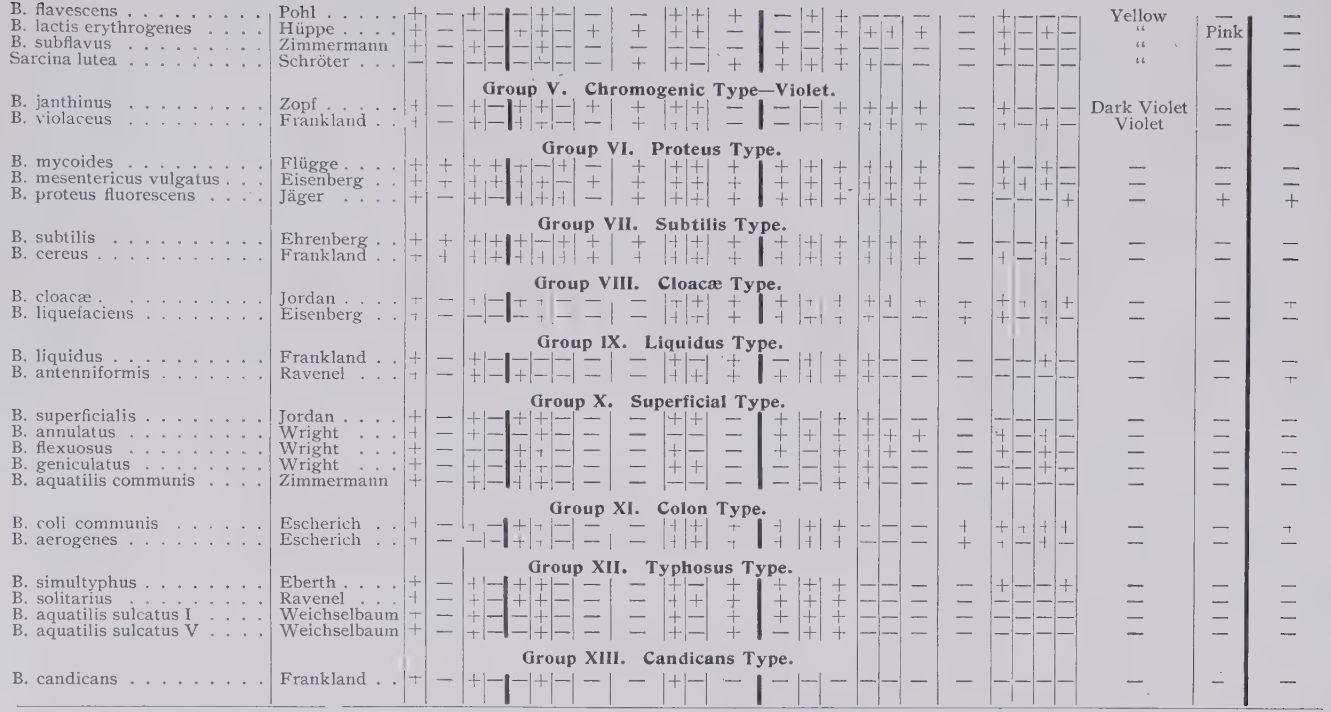
* "Amer. Jour. Med. Sci.," 1895, 110, p. 301.

† "Public Health and Journal of Experimental Medicine."

water, the report of the Committee on Standard Methods of Water Analysis to the Laboratory Section of the American Public Health Association, published in Supplement No. 1 of the "Journal of Infectious Diseases," May, 1905, will prove indispensable.

CLASSIFICATION OF BACILLI FOUND IN OHIO RIVER WATER AT CINCINNATI, OHIO.

Name of Organism.	First Investigator.	Morphology.				Biology.																						Pathogenesis
						Cultural Features.									Biochemical Features.													
						Nutrient Broth Tube.		Nutrient Agar Tube.	Gelatin Plate.		Potato Tube.		Fermentation Tube.					Liquefaction.		Gas Production.					Nutrient Agar Tube.		White Mice.
		Bacillus.	Diameter Greater than 1 μ.	Motile.	Spores.	Scum.	Turbid.	Dull.	Wrinkled.	Characteristic Appearance.	Visible.	Luxuriant.	Growth in Closed Arm.	Grows at Body Temperature.	Facultative Anaerobe.	Affected by Range of Reaction.	Gelatin.	Casein.	Blood-serum.	Dextrose Broth.	Nitrate Reduced.	Indol Produced.	Milk Coagulated.	Fecal Odor.	Chromogenesis.	Fluorescence.	Intraperitoneal Inoculation Fatal.
Group I. Fluorescent Type.																												
B. fluorescens liquefaciens	Flügge	+	—	+	—	+	+	—	—	+	+	—	—	—	—	+	+	+	+	—	+	—	—	—	—	—	+	—
B. fluorescens non-liquefaciens	Eisenberg	+	—	—	—	—	+	+	—	—	+	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	+	—
B. viridis	Lesage	+	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+
B. fluorescens ovalis	Ravenel	+	—	+	—	+	+	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+
B. pyocyaneus	Gessard	+	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+
B. fluorescens incognito	Wright	+	—	+	—	+	+	—	—	—	+	+	—	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+
Group II. Chromogenic Type—Red.																												
B. prodigiosus	Ehrenberg	+	—	+	—	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	—	+	—	Crimson	—	—	—
B. rubidus	Eisenberg	+	—	+	—	+	+	—	—	+	+	—	+	—	+	+	+	+	+	+	+	—	—	—	Brick Red	—	—	—
Group III. Chromogenic Type—Orange.																												
B. arborescens	Frankland	+	—	+	—	—	+	+	—	+	+	—	—	—	+	+	+	+	—	—	—	—	—	—	Orange	—	—	—
B. aurescens	Ravenel	+	—	+	—	—	—	+	—	—	+	—	—	+	—	+	+	—	+	—	—	—	+	—	"	—	—	—
B. fulvus	Zimmermann	+	—	—	—	—	+	—	+	+	+	—	+	—	+	+	—	—	—	—	—	—	—	—	"	—	—	—
B. fuscus	Zimmermann	+	—	—	—	—	—	+	—	—	+	—	+	+	+	+	—	—	—	—	—	—	—	—	"	—	—	—
B. aurantiacus	Frankland	+	—	+	—	—	+	—	—	+	+	—	—	—	—	+	—	—	—	—	+	—	—	—	"	—	—	—
Group IV. Chromogenic Type—Yellow.																												
B. desidious	Wright	+	—	—	—	+	+	—	—	—	+	+	—	—	—	+	+	+	+	—	—	—	+	—	Yellow	—	—	—
B. ochraceus	Zimmermann	+	+	+	—	—	—	—	+	—	+	—	—	+	—	+	+	—	+	—	+	—	+	—	"	—	—	—



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CHAPTER XIV.

BACTERIOLOGY OF THE SOIL.

The upper layers of the soil contain bacteria in proportion to their richness in organic matter. Near the habitations of men, where the soil is cultivated, the excrement of animals, largely made up of bacteria, is spread upon it to increase its fertility, this treatment not only adding new bacteria to those already present, but also enabling those present to grow much more luxuriantly because of the increased nourishment they receive.

Where, as in Japan, human excrement is used to fertilize the soil, or as in India, it is carelessly deposited upon the ground, bacteria of cholera, dysentery, and typhoid fever are apt to become disseminated by fresh vegetables, or through water into which the soil drains. In such localities fresh vegetables should not be eaten, and water for drinking should be boiled.

The researches of Flügge, C. Fränkel, and others show that the bacteria of the soil do not penetrate deeply, but gradually decrease in number until the depth of a meter is reached, then rapidly diminish until at a meter and a quarter they rather abruptly disappear.

The bacteria of soil are, for the most part, harmless saprophytes, though a few highly pathogenic organisms, such as the bacilli of tetanus and malignant edema, occur. Many of them are anaerobic, and it is interesting to speculate upon their biology. Whether they develop and multiply in the soil in intimate association with strongly aerobic organisms by which the free oxygen is absorbed, or whether they remain latent in the soil and develop only in the intestines of animals, is not known.

The estimation of the number of bacteria in the soil seems to be devoid of any practical importance. C. Fränkel has, however, originated an accurate method of determining it. By means of a special boring apparatus (Fig. 71) earth can be secured from any depth without digging and without danger of mixing with that of the superficial strata. A

measured quantity of the secured soil is thoroughly mixed with liquefied sterile gelatin and poured into a Petri dish or solidified upon the walls of an Esmarch tube. The colonies are counted with the aid of a lens. Flügge found in virgin earth about 100,000 colonies in a cubic centimeter.

Samples of earth, like samples of water, should be examined as soon as possible after being secured, for, as Günther points out, the number of bacteria changes because of the unusual dryness, warmth, exposure to oxygen, etc.

The most important bacteria of the soil are those of tetanus and malignant edema, in addition to which, however, there are a great variety of organisms pathogenic for rabbits, guinea-pigs, and mice.

Fig. 71.—Tip of Fränkel's instrument for obtaining earth from various depths for bacteriologic study. *B* shows the instrument with its cavity closed, as it appears during boring; *A*, open, as it appears when twisted in the other direction to collect the earth.

In the "Bacteriological Examination of the Soil of Philadelphia," Ravenel * came to the conclusion that—

1. Made soils, as commonly found, are rich in organic matter and excessively damp through poor drainage.

2. They furnish conditions more suited to the multiplication of bacteria than do virgin soils, unless the latter are contaminated by sewage or offal.

3. Made soils contain large numbers of bacteria per gram of many different species, the deeper layers being as rich in the number and variety of organisms as the upper ones. After some years the number in the deeper layers probably becomes proportionally less. Made soils are more likely than others to contain pathogenic bacteria.

In seventy-one cultures that were isolated and carefully studied by Ravenel, there were two cocci, one sarcina, and five cladothrices; all the others were bacilli.

* "Memoirs of the National Academy of Sciences," First Memoir, 1896.

CHAPTER XV.

THE BACTERIOLOGY OF FOODS.

THE relation of bacteria to foods is an important one and should be as thoroughly understood as possible by both the profession and the laity. The relationship may be expressed thus:

I. Foods serve as vehicles by which infectious agents are conveyed to the body.

II. Foods are chemically changed and made unfit for use by the bacteria.

I. Foods as Fomites.—In animal food the first source of infection is the animal itself, danger of infection always accompanying the employment of foods derived from diseased animals. Thus, milk apparently normal in appearance has been found to contain dangerous pathogenic bacteria. The tubercle bacillus is one of the most important of these, and at the present time the consensus of opinion inclines toward the view that the great prevalence of tuberculosis among human beings depends partly upon the ingestion of tubercle bacilli in milk. It does not appear necessary that the udder of the cow be diseased in order that the organisms enter the milk, as they seem to have been found in milks derived from cows whose udders were entirely free from demonstrable tuberculosis. It is, therefore, imperative to retain only healthy cows in the dairy, and careful legislation should provide for the detection and destruction of all tuberculous animals. The detection of tubercle bacilli in milk can only be certainly accomplished by the injection of a few cubic centimeters of the fluid into guinea-pigs and noting the results.

In addition to the tubercle bacillus, pyogenic streptococci have been observed in enormous quantities and almost pure culture in milk drawn from cows suffering from mastitis. Stokes * has observed a remarkable case of this kind in which the milk contained so much pus that it floated upon the top

* "Maryland Medical Journal," Jan. 9, 1897.

like cream. Such seriously infected milk could not be used with safety to the consumer.

In market milk one occasionally finds pathogenic organisms, such as the diphtheria bacillus, typhoid bacillus, streptococcus, etc., derived from human sources. Such polluted milks have been known to spread epidemics of the respective diseases whose micro-organisms are present. Bacteria may enter milk from careless handling, from water used to wash the cans or to dilute the milk, or from dust; and as milk is an excellent medium for the growth of bacteria, it should always be treated with the greatest care to prevent such contamination, as saprophytic bacteria produce chemical changes in the milk, such as acidity and coagulation, which destroy its usefulness or render it dangerous as food for infants and invalids. Where the necessary precautions are not or cannot be taken, Pasteurization of the milk as soon after its reception as possible may act as a safeguard.

Meat from tuberculous animals might cause disease if eaten raw or but partially cooked. As cooking suffices to kill the organisms, the danger under ordinary conditions is not great. Moreover, tuberculosis rarely affects the muscles, the parts usually eaten.

Butter made from cream derived from tuberculous milk may also contain tubercle bacilli, as has been shown by the researches of Rabinowitsch.*

Foods may become polluted with bacteria in a variety of ways that will suggest themselves to the reader. The common source is dust, which is more or less rich in bacteria according to the soil from which it arises. The readiness with which raw foods, such as meats, milk, etc., can be thus contaminated in the barnyard, dairy, slaughter-house, and shop, teaches but one lesson—that the greatest cleanliness should prevail for the sake of the dealer, whose goods may be spoiled by carelessness, and the consumer, who may be injured by the food.

Any food may carry infectious organisms upon its surface, such organisms being derived from the hands of the dealer, from dust, from water, as when green vegetables are sprinkled with impure water to keep them fresh, or from other sources.

The cleanliness of the merchant and the protection from

*"Deutsche med. Wochenschrift," 1900, No. 26; abstract in the "Centralbl. f. Bakt.," etc., XXIX, 1901, p. 309.

contamination that he bestows upon his goods should be taken into consideration by his customers.

Shell-fish, especially oysters, seem to be common carriers of infection, especially of typhoid fever. The oysters seem to be contaminated with infected sewage carried to their beds. It is not yet satisfactorily determined whether typhoid bacilli multiply in the juices in the shells of the oysters or not, but a number of epidemics of typhoid fever have been very conclusively traced to the consumption of certain oysters at a definite time and place. As cooking the oysters will kill the contained bacilli, the prophylaxis of disease in this case is very simple.

II. Food Poisons.—A new and useful nomenclature, suggested by Vaughan and Novy,* contains the following terms:

Bromatotoxismus—food-poisoning;
Galactotoxismus—milk-poisoning;
Tyrotoxismus—cheese-poisoning;
Kreotoxismus—meat-poisoning;
Ichthyotoxismus—fish-poisoning;
Mytilotoxismus—mussel-poisoning;
Sitotoxismus—cereal-poisoning.

The most important chemic alterations effected by bacteria occur in milk and meat.

1. Milk-poisoning (*Galactotoxismus*).—Milk, even when freshly drawn from the cow, always contains some bacteria, whose numbers gradually diminish for a few hours, then rapidly increase until almost beyond belief. These organisms are for the most part harmless to the consumer, but ultimately ruin the milk. Although much attention has been paid to the subject, bacteriologists are not agreed whether the number of bacteria contained in milk is a satisfactory guide as to its harmfulness.

The poisonous change in milk, cream, ice-cream, etc., has been shown by Vaughan to depend in part upon the presence of a ptomain known as *tyrotoxicon*, formed by the growth of bacteria in the milk, but whether of any particular bacterium is not known. The milk may become poisonous during any time of the year, but chiefly in the summer, when, because of the higher temperature, bacteria develop most rapidly. The change takes place in stale milk,

*"Cellular Toxins," Phila., 1902.

and it is supposed that many cases of what was formerly looked upon as "summer complaint" in infants were really poisoning by this toxic ptomain.

Ice-cream poisoning depends upon the growth of the bacteria in the milk before it is frozen. In some cases the error made has been to prepare the cream for freezing and then keep or transport it, the freezing operation being delayed until the development of the bacteria has led to the poisonous condition.

Cheese-poisoning (*Tyrotoxismus*) is also thought to depend upon tyrotoxicon at times, though it has been shown that other cheese poisons exist. It is more or less a question whether cases of milk- and cheese-poisoning do not depend upon the toxic products of the colon bacillus growing in the foods.

2. **Meat-poisoning** (*Kreotoxismus*).—Botulism or meat-poisoning depends upon the growth of certain bacteria, Bacillus botulinus of van Ermengem,* in the meat. The symptoms following infection by the organism sometimes closely resemble those of typhoid fever, and are characterized by acute gastro-intestinal irritation, nervous disturbances, and, in case of death, by fatty degenerations in the organs and minute interstitial hemorrhages.

3. **Fish-poisoning** (*Ichthyotoxismus*) sometimes follows the consumption of canned and presumably spoiled fish, sometimes the consumption of diseased fish. It is not known whether it depends upon ptomains or upon toxicogenic germs, though probably the latter, as Silber has isolated a Bacillus piscicidus that is highly toxicogenic.

4. **Mussel-poisoning** (*Mytilotoxismus*) depends partly upon irritating and nervous poisons in the mussel substance, in part upon toxicogenic germs that they harbor.

5. **Canned Goods.**—Improperly preserved canned goods not infrequently spoil because of the growth of bacteria, but the occurrence of gas-formation, acidity, insipidity, etc., causes rejection of the product, and but few cases of poisoning from canned goods can be authenticated.

* "Zeitschrift für Hygiene," Bd. xxvi, Heft 1.

CHAPTER XVI.

THE DETERMINATION OF THE THERMAL DEATH-POINT OF BACTERIA.

SEVERAL methods may be employed for this purpose. Roughly, it may be done by keeping a bouillon culture of the micro-organism to be investigated in a water-bath whose temperature is gradually increased, transplantations being made from time to time until the temperature fatal to the bacteria is reached.

It is economy to make the transplantations less frequently at first than later in the experiment, when the ascending temperature approaches a height dangerous to life. In ordinary determinations it is well to make a transfer at 40° C., another at 45°, another at 50°, still another at 55°, and then, beginning at 60°, make one for every additional degree. The day following the experiment it will be observed that all the cultures grow except those heated beyond a certain point, say 62° C., when it can properly be concluded that 62° C. is the thermal death-point. If all the transplantations grow, of course the maximum temperature was not reached, and the experiment must be repeated and the bacteria exposed to still higher temperatures.

When more accurate information is desired, and one wishes to know how long the micro-organism can endure some such temperature as 60° C. without losing its vitality, a dozen or more bouillon-tubes may be inoculated with the organism to be studied, and stood in a water-bath kept at the temperature to be investigated. The first can be removed as soon as it is heated through, another in five minutes, another in ten minutes, or at whatever intervals the thought and experience of the experimenter shall suggest, the subsequent growth in each culture showing that the endurance of the organism had not yet been exhausted. By using gelatin and pouring each culture into a Petri dish, and subsequently counting the colonies, it can be determined whether many or only a few of the organisms in a culture

possess the maximum resisting power. To determine the percentage, it is necessary to know how many bacteria were present in the tubes before exposure to the destructive temperature. Approximately the same number can be placed in each tube by adding the same measured quantity of a fluid culture to each.

In both of the procedures one must be careful that the temperature of the fluid in the test-tube is identical with that of the water in the bath. A sterile thermometer introduced into an uninoculated tube exposed under conditions similar to those of the experiment can be used as an index for the others.

Another method of accomplishing the same end is by the use of Sternberg's bulbs. These are small glass bulbs blown on one end of a glass tube, drawn out to a fine point at the opposite end. If such a bulb be heated so that the air is expanded and partly driven out, its open tube, dipped into inoculated bouillon, will in cooling draw the fluid in, so as to fill it one-third or one-half. A number of these tubes are filled in this manner with a freshly inoculated culture medium and then floated, tube upward, upon a water-bath whose temperature is gradually elevated, the bulbs being removed from time to time as the required temperatures are reached. As the bulbs are already inoculated, all that is necessary is to stand them aside for a day or two, and observe whether or not the bacteria grow, determining the death-point exactly as in the other case.

CHAPTER XVII.

DETERMINATION OF THE VALUE OF ANTISEPTICS, GERMICIDES, AND DISINFECTANTS.

The student must bear in mind that an antiseptic is a substance capable of restraining the growth of bacteria; a germicide, one capable of killing them. All germicides are antiseptic in dilute solutions, but not all antiseptics are germicides. Disinfectants must be germicides.

Antiseptics are chiefly employed for purposes of preservation, and are largely used in the industries to protect organic substances from the micro-organisms of fermentation and decomposition. The problem is to secure a satisfactory effect with the addition of the least possible preservative in order that its presence shall not chemically destroy the good qualities of the substances preserved. In the case of foods it becomes necessary to use preservatives free from poisonous properties.

Disinfectants and germicides are employed for the purpose of destroying germs of all kinds, and the chief problem is to secure efficiency of action, rather than to endeavor to save on the reagent, which would be a false economy, in that the very object desired might be defeated.

The following methods of determining the antiseptic and germicidal values of various agents can be elaborated according to the extent and thoroughness of the investigation to be made.

I. The Antiseptic Value.—Remembering that an antiseptic is a substance that inhibits bacterial growth, the determination of its value can be made by adding varying quantities of the antiseptic to be investigated to culture media in which bacteria are subsequently planted. It is always well to use a considerable number of tubes of bouillon containing varying strengths of the reagent to be investigated. If the antiseptic be non-volatile, it may be added before sterilization, which is to be preferred; but if volatile, it must be added by means of a sterile pipet, with the greatest

precaution as regards asepsis, after sterilization and immediately before the test is made. Control experiments—*i. e.*, bouillon cultures without the addition of the antiseptic—should always be made.

The results of antiseptic action are two: *retardation* of growth and *complete inhibition* of growth. As the inoculated tubes containing the antiseptic are watched in their development, it will usually be observed that those containing very small quantities develop almost as rapidly as the control tubes; those containing more, a little more slowly; those containing still more, very slowly, until at last there comes a time when the growth is entirely checked.

Sternberg points out that the following conditions, which must be avoided, may modify the results of experiment:

1. The composition of the nutrient media, with which the antiseptic may be incompatible (as bichloride of mercury and albumin).

2. The nature of the test-organism, no two organisms being exactly alike in their susceptibility.

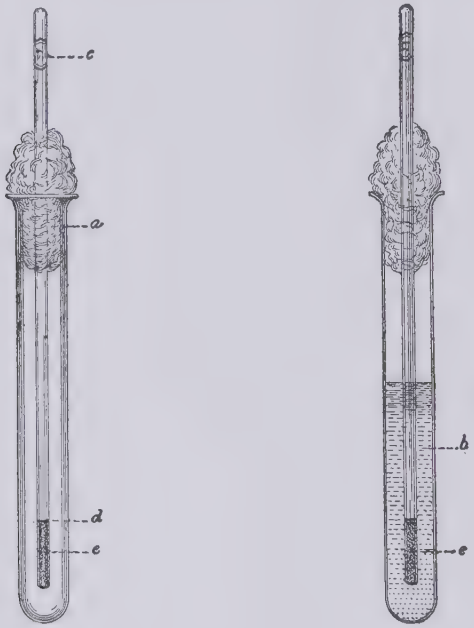
3. The temperature at which the experiment is conducted, a relatively greater amount of the antiseptic being necessary at temperatures favorable to the organism than at temperatures unfavorable.

4. The presence of spores which are always more resistant than the asporogenous forms.

II. The Germicidal Value.—Koch's original method of determining this was to dry the micro-organisms upon sterile threads of linen or silk, and then soak them for varying lengths of time in the germicidal solution. After the bath in the reagent the threads were washed in clean, sterile water, transferred to fresh culture media, and their growth or failure to grow observed. This method also determines the *time* in which a certain solution will kill micro-organisms, so is advantageous.

Sternberg suggested a method by which the *dilution* necessary to kill the bacteria could be determined, the time remaining constant (two hours' exposure) in all cases. "Instead of subjecting test-organisms to the action of the disinfecting agent attached to a silk thread, a certain quantity of a recent culture—usually 5 c.c.—is mixed with an equal quantity of a standard solution of the germicidal agent, . . . and after two hours' contact one or two loopfuls are transferred to a suitable nutrient medium to test the question of disinfection."

A very simple and popular method of determining the germicidal value is to make a series of dilutions of the reagent to be tested; add to each a small quantity of a fresh liquid culture, and at varying intervals of time transfer a loopful to fresh culture media. By a little ingenuity this



Same rod immersed in broth after exposure to disinfectant.

Fig. 72.—Glass rod in test-tube, for use in testing disinfectants. Tube 6 in. by $\frac{3}{4}$ in.; rod 9 in. by $\frac{1}{4}$ in. Ring marked with diamond 1 in. from lower end, to show upper limit of area on which the organisms are dried. After exposure the rod is placed in a similar tube containing broth, to test development. *a*, Cotton plug wrapped around glass rod; *b*, broth; *c*, gummed label on handle of rod, for indentification; *d*, ring marked by diamond; *e*, dried organisms.

method may be made to yield information as to both *time* and *strength*.

Hill * has suggested a convenient method of handling the cultures, which are dried upon the ends of sterile glass rods and can then be transferred from one solution to another or otherwise manipulated (see Fig. 72).

* "Public Health," vol. XXIV, p. 246.

When it is desired to secure information concerning the progress of the germicidal action of reagents, body-fluids, etc., especially in the unusual and interesting cases in which the material subjected to the test may exert a restraining action for a time only, or bring about destruction of some or many, but not all of the germs, it is imperative to plate the culture and count the colonies. For this purpose the Petri dish can be used with advantage.

For example, if it be desired to determine whether blood serum be germicidal or not, the following method can be employed. Into about 5 c.c. of the serum contained in a test-tube, two or three loopfuls of any desired bacterium, in liquid culture, are added. The tube is well agitated and immediately one loopful is transferred to a tube of melted gelatin, distributed through it, and poured into a Petri dish. After one minute the operation is repeated, in five minutes again, and so on as often as is desired.

The dishes are stood away until the colonies of bacteria develop and can be counted with a Wolfhügel or other apparatus. On the first dish there may be 100 colonies; on the second, 80; on the third, 50; on the fourth, 20; on the fifth, 30; on the sixth, 150; on the seventh, 1000, etc.; indicating that the serum exerted a destructive action upon some, but not all, of the bacteria, and that this power disappeared after the lapse of a certain time, allowing the bacteria to develop *ad libitum*.

Control experiments are indicated in this kind of work, as the very fact that the bacteria are transferred from one medium to another is commonly sufficient to cause the death of a large number.

Gaseous Disinfection.—If the germicide to be studied be a gas, as in the case of sulphurous acid or formaldehyd, a different method must, of course, be adopted.

It may be sufficient to place a few test-tube cultures of various bacteria, some with plugs in, some with plugs out, in a closed chamber in which the gas is evolved. The germicidal action is shown by the failure of the cultures to grow upon transplantation to fresh culture media. This crude method may be supplemented by an examination of the dust of the room. Pledgets of sterile cotton are rubbed upon the floor, washboard, or any dust-collecting surface present, and subsequently dropped into culture media. Failure of growth under such circumstances is very certain evidence of good

disinfection. These tests are, however, very severe, for in the cultures there are immense numbers of bacteria in the deeper portions of the bacterial mass upon which the gas has no opportunity to act, and in the dust there are many sporogenous organisms of extreme resisting power. Failure to kill all the germs exposed in such manner is no indication that the vapor cannot destroy all the ordinary pathogenic organisms.

A more refined method of making the tests consists in saturating strips of blotting-paper, absorbent cotton, various fabrics, etc., with cultures and exposing them, moist or dry, to the action of the gas. Such materials are best made ready in Petri dishes, which are opened immediately before and closed immediately after the experiment. If, when transferred to fresh culture media, the exposed objects fail to give any growth, the disinfection has been thorough. If the penetrating power of a gas, such as formaldehyd, is to be tested, it can be done by inclosing the infected paper or fabrics in envelopes, boxes perforated with small holes, tightly closed pasteboard boxes, and by wrapping them in towels, blankets, mattresses, etc.

Easier of execution, but rather more severe, is a method in which cover-glasses are employed. A number of them are sterilized, spread with cultures of various bacteria, allowed to dry, and then exposed to the gas as long as required. They are subsequently dropped into culture media to permit the growth of the organisms not destroyed.

Animal experiments may also be employed to determine whether or not a germ that has survived exposure to the action of reagents has its pathogenic power destroyed. An excellent example of this is seen in the case of the anthrax bacillus, a virulent form of which will kill rabbits, but after being grown in media containing an insufficient amount of a germicide to kill it will often lose its rabbit-killing power, though still able to fatally infect guinea-pigs, or may lose its virulence for both rabbits and guinea-pigs, though still able to kill white mice.

PART II. SPECIFIC DISEASES AND THEIR BACTERIA.

(A) THE PHLOGISTIC DISEASES.

I. THE ACUTE LOCAL INFECTIVE DISEASES.

CHAPTER I.

WOUND INFECTION; SUPPURATION.

SUPPURATION was at one time looked upon as a normal and inevitable outcome of the majority of wounds, and although bacteria were early observed in the purulent discharges, the insufficiency of information then at hand led to the belief that they were spontaneously developed there.

From what has already been said about the evolution of bacteriology and the biology and distribution of bacteria, the relationship existing between bacteria and suppuration, and indeed between bacteria and disease in general, is found to be reversed. Instead of being the products of disease, we now look upon the bacteria as the cause.

With this altered point of view came the question, Whence come the bacteria that cause disease? The wide distribution of bacteria in the air naturally led surgeons to look upon it as the source of all infection, and to make most strenuous, though mistaken, efforts to disinfect it, that it might not contaminate wounds.

The development of antiseptic surgery, and the extremes to which the application of germicides was carried, became almost ridiculous, for not only were the hands of the operator, his instruments, sponges, sutures, ligatures, and dressings kept constantly saturated with powerful and irritating

germicidal solutions, and the wound subsequently covered by dressings saturated with germicides, but by means of a steam atomizer the air over the wound was kept filled with a disinfecting vapor during the whole operation.

More recent researches, however, have shown not only that the atmosphere cannot be disinfected, but also that the air of ordinarily quiet rooms, while containing a few saprophytic organisms, very rarely contains pathogenic bacteria, and is rarely an inportant factor in wound infection. A direct stream of air, such as is generated by an atomizer, really directs more bacteria toward the wound than would ordinarily fall upon it, thereby increasing, instead of lessening, the danger of infection.

The strong disinfecting solutions once employed have likewise been largely abandoned, the modern view being that it is far easier to prevent the entrance of organisms into wounds than to destroy them by the application of strong and irritating solutions.

Suppuration, while nearly always the result of micro-organismal activity, is not a specific infectious process, but the expression of a violent tissue-reaction that may result from various injurious agents.

Being, therefore, but the expression of tissue irritation associated with strong chemotactic influences, it is only to be expected that as many bacteria may be associated with it as can bring about the essential conditions. Bacteria with which these qualities are exceptionally marked appear as the common cause of the process; those with which it is less marked, as exceptional causes.

Attention has already been called to the fact that certain micro-organisms are so intimate in their relation to the skin that it is almost impossible to get rid of them, and in this relation the experiments of Welsh, Robb, and Ghriskey upon hand disinfection have been cited. These observers have shown that, no matter how rigid the disinfection of the patient's skin, the cleansing of the operator's hands, the sterilization of the instruments, and the precautions exercised, a certain number of wounds in which sutures are employed will always suppurate, the cause of the suppuration being the skin cocci, all of which it is impossible to remove. We thus carry infectious organisms constantly with us upon our skins, and so pave the way for suppuration in wounds. That all wounds do not suppurate probably depends largely

upon the local and general immunity of the individual, rather than upon the absence of organisms from the wounds.

Staphylococcus Epidermidis Albus (Welch).

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, slowly liquefying, non-chromogenic, aerobic and optionally anaerobic, doubtfully pathogenic coccus, staining by the usual methods and by Gram's method, and having its natural habitat upon the skin.

Under the name *Staphylococcus epidermidis albus*, Welch * has described a micrococcus which seems to be habitually present upon the skin, not only upon the surface, but also deep down in the Malpighian layer. He believes it to be Staphylococcus pyogenes albus in an attenuated condition, and if this opinion be correct, and there is seated deeply in the derm a coccus which may at times cause suppuration, the conclusions of Robb and Ghriskey, that sutures of catgut when tightly drawn may be a cause of skin-abscesses by predisposing to the development of this organism, are certainly justifiable. As all the morphologic and cultural characteristics of the organism correspond to those of the following species, no separate description of them seems necessary.

Staphylococcus Pyogenes Albus (Rosenbach†).

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, liquefying, non-chromogenic, aerobic and optionally anaerobic, mildly pathogenic coccus, staining by the ordinary methods and by Gram's method.

Although, as stated, Staphylococcus pyogenes albus is a common cause of suppuration, it rarely occurs alone, Passet so finding it in but 4 out of 33 cases investigated. When pure cultures of the coccus are subcutaneously injected into rabbits and guinea-pigs, abscesses occasionally result. Injected into the circulation, the staphylococci occasionally cause septicemia, and after death can be found in the capillaries, especially in the kidneys. From this it will be seen that the organism is feebly and variably pathogenic.

In its morphologic and vegetative characteristics Staphylococcus albus is almost identical with the species next to be described, differing from it only in the absence of its characteristic golden pigment.

* "Amer. Jour. Med. Sci.," 1891, p. 439.

† "Wundinfektionskrankheiten des Menschen," Wiesbaden, 1884.

Staphylococcus Pyogenes Aureus (Rosenbach *).

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, liquefying, chromogenic, pathogenic, aerobic and optionally anaerobic coccus, staining by the ordinary methods and by Gram's method.

Commonly present upon the skin, though in smaller numbers than the organisms already described, is the more virulent and sometimes dangerous *Staphylococcus pyogenes aureus* (Fig. 73), or "golden staphylococcus," first observed by Ogston and cultivated by Rosenbach. As the morphology and cultural characteristics of this organism are identical with those of the preceding species, it seems convenient to describe them together, pointing out such minor differences as occur. In doing this, however, it must

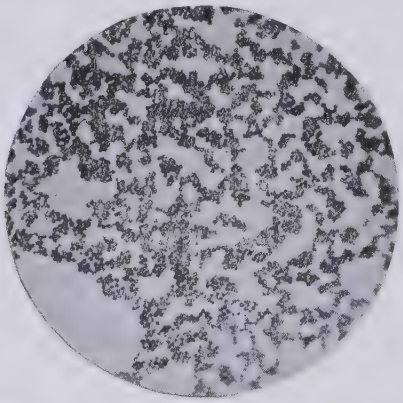


Fig. 73.—Staphylococcus pyogenes aureus, from an agar-agar culture. × 1000 (Günther).

not be forgotten that, although Staphylococcus albus was first mentioned, Staphylococcus aureus is the more common organism of suppuration.

Staphylococci Pyogenes Aureus et Albus.

Distribution.—The cocci are not widely distributed in nature, seeming not to find a purely saprophytic existence satisfactory. They occur, however, upon man and the lower animals, and can occasionally be found in the dusts of

* "Mikroorganismen bei Wundinfektionskrankheiten des Menschen," Wiesbaden, 1884.

houses and hospitals,—especially in the surgical wards if proper precautions are not exercised. They are common upon the skin, in the nose, mouth, eyes, and ears of man; they are nearly always present beneath the finger-nails, and they sometimes occur in the feces, especially of children.

Morphology.—The cocci are small, measuring about 0.7 μ in diameter. When properly stained, the organisms are found to consist of hemispheres separated from one another by a narrow interval, the approximated surfaces being flattened. As observed in hastily stained preparations, they are spheric. There is no definite grouping in either liquid or solid cultures. It is only in pus or in the organs or tissues of diseased animals that one can say that a true staphylococcus grouping occurs.

The organisms are not motile and have no flagella.

Staining.—The organisms stain easily and brilliantly with aqueous solutions of the anilin dyes and by Gram's method.

Isolation.—Staphylococcus aureus is an easy organism to isolate, and can be secured by plating out a drop of pus in gelatin or in agar-agar. Such preparations, however, generally do not contain Staphylococcus aureus by itself, but in association with Staphylococcus albus.

As the colonies of Staphylococcus aureus differ considerably in color, some being much paler than others, I have often doubted whether Staphylococcus albus was a different species, or simply a non-chromogenic form of pus coccus. It is possible to secure nearly every intermediate tint from white to golden yellow by a little manipulation. Should this be the case, it would reduce the pus cocci to a single species, Staphylococcus pyogenes.

Cultivation.—The staphylococci grow well upon all the standard culture media either in the presence or in the absence of oxygen at temperatures above 18° C., the most rapid development being at about 37° C.

Colonies.—Upon the surface of gelatin plates the colonies appear as small whitish points after from twenty-four to forty-eight hours (Fig. 74), rapidly extending to the surface and causing extensive liquefaction of the medium. The formation of the orange pigment can be best observed near the center of the colonies. Under the microscope the colonies appear as round disks with circumscribed, smooth

edges. They are distinctly granular and dark brown. When the colonies are grown upon agar-agar plates, the formation of the pigment is more distinct.

Gelatin Punctures.—In gelatin the growth occurs along the whole length of the puncture, causing an extensive liquefaction of the medium in the form of a long, narrow, blunt-pointed, inverted cone, sometimes described as being like a stocking (Fig. 75), full of clouded liquid, at the apex of which a collection of golden or orange-yellow precipitate is always present in Staphylococcus aureus. It is this precipitate in particular that gives the organism its name "golden staphylococcus."

Agar-agar.—The growth of the golden staphylococcus

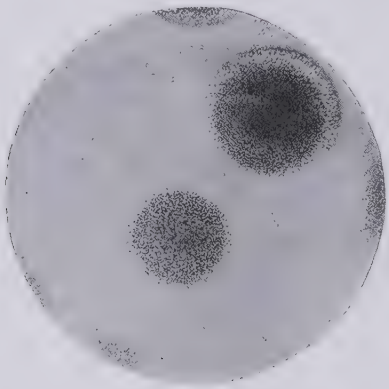


Fig. 74.—Staphylococcus pyogenes aureus. Colony two days old, seen upon an agar-agar plate. × 40 (Heim).

upon agar-agar is subject to considerable variation in the quantity of pigment produced. Sometimes, perhaps rarely, it is golden; more commonly it is yellow, often cream color. Along the whole line of inoculation a moist, shining, usually well-circumscribed growth occurs. When the development occurs rapidly, as in the incubator, it exceeds the rapidity of color-production, so that the center of the growth is distinctly colored, the edges remaining white.

Potato.—Upon potato the growth is luxuriant, Staphylococcus aureus producing an orange-yellow coating over a large part of the surface. The potato cultures may give off a sour odor.

Bouillon.—When grown in bouillon, the organism causes

a diffuse cloudiness, with a small quantity of slightly yellowish sediment.

Milk.—In milk coagulation takes place and is followed by gradual digestion of the casein.

Toxic Products.—Leber seems to have first conceived of suppuration as a toxic process depending upon the soluble products of parasitic fungi, and in 1888, through the action of alcohol upon staphylococci, prepared an acicular crystalline body soluble in alcohol and ether, but slightly soluble in water, to which he gave the name *phlogosin.*

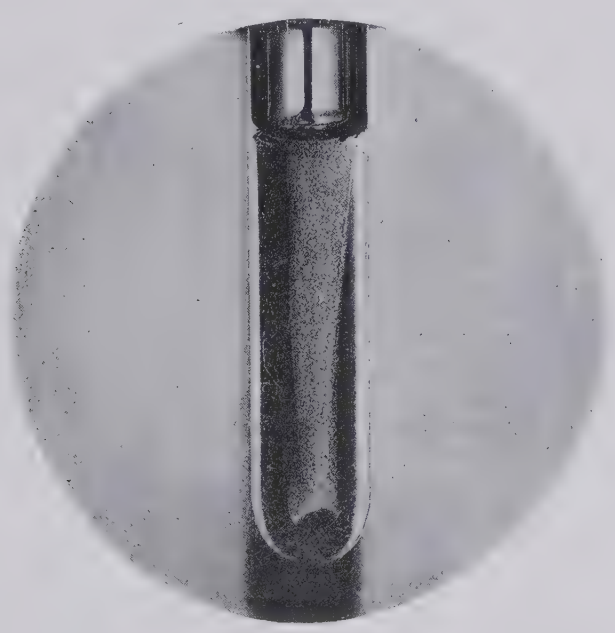


Fig. 75.—Staphylococcus pyogenes aureus. Puncture culture three days old in gelatin (Fränkel and Pfeiffer).

Mannatti found that pus has substantially the same toxic properties as sterilized cultures of the staphylococcus; that repeated injections of sterilized pus induce chronic intoxication and marasmus; that injection of sterilized pus under the skin causes a grave form of poisoning; and that the symptoms and pathologic lesions caused by these injections correspond with those observed in men suffering from chronic suppuration.

Van de Velde* found that the staphylococcus has some metabolic products destructive to the leukocytes, which he has called *leukocidin.* This poison causes the cells to cease ameboid movement, become spheric, and gradually to lose thin granules, until they finally appear like empty sacs containing shadow nuclei, which eventually disappear. The leukolysis occurs in about two minutes. These observations have been abundantly confirmed. Krauss† first observed that certain products of the staphylococcus were hemolytic and destroyed red blood-corpuscles. This hemolysin has been carefully studied by Neisser and Wechsberg,‡ by whom it was called *staphylolysin.*

Durme§ found staphylolysin produced most abundantly by virulent staphylococci.

Simon‖ found that the toxic quality of the bodies of streptococci of different stocks had nothing to do with their virulence. Simon** also found that the toxic products of the streptococcus were diverse and peculiar. The bodies of the cocci contained an intracellular toxin the activity of which was independent of their virulence. This poison is liberated only when the bactericidal activities of the body act upon the cocci. The cocci also excrete a toxic substance whose activity is greater than that of the intracellular toxin, but whose production is subject to great variation and is entirely independent of the intracellular toxin. The toxins and hemolysins are entirely different bodies.

Ribbert†† found that both sterilized and unsterilized cultures when intravenously injected into animals produced definite changes in the heart, kidneys, lungs, spleen, and bone-marrow, and attributed the action to the toxin.

Morse‡‡ found that the toxic products of Staphylococcus aureus were capable of occasioning interstitial nephritis.

The staphylococci form very little extracellular toxin, as

*"La Cellule," XI, 1896, p. 349.

†"Wiener klin. Wochenschrift," 1900.

‡"Zeitschrift für Hygiene," 1901, XXXVI, p. 330.

§"Hyg. Rundschau," 1903, Heft 2, p. 66.

‖"Centralbl. f. Bakt.," etc., XXXV, No. 3, p. 308, Dec. 18, 1903.

**"Centralbl.," Jan. 16, 1904, XXXV, No. 4, p. 350.

††"Die pathologische Anatomie und die Heilung der durch den Staphylococcus pyogenes aureus hervorgerufenen Erkrankungen."

‡‡"Journal of Experimental Medicine," vol. I, 1896, p. 613.

filtered cultures provoke little local or general reaction in animals, even when the staphylococcus is highly virulent.

Pathogenesis.—The virulent, golden staphylococcus is a dangerous and often deadly organism. When introduced subcutaneously, abscesses commonly result and not infrequently lead to a fatal generalization of the organisms. In such cases the organisms may be cultivated from the streaming blood, though the greater number collect in, and frequently obstruct, the capillaries. In the lungs and spleen, and still more frequently in the kidneys, infarcts are formed by the bacterial emboli. The Malpighian tufts of the kidneys are sometimes full of cocci, and become the centers of small abscesses.

The coccus is almost equally pathogenic for man and the lower animals, though the fatal outcome of human infection is more rare, possibly because of the conditions of infection. It enters the human system through scratches, punctures, or abrasions, and when virulent usually occasions an abscess. Garré applied the organism in pure culture to the uninjured skin of his arm, and in four days developed a large carbuncle with a surrounding zone of furuncles. Bockhart suspended a small portion of an agar-agar culture in salt solution, and scratched it gently into the deeper layer of the skin with his finger-nail; a furuncle developed. Bumm injected the coccus suspended in salt solution beneath his skin and that of several other persons, and produced an abscess in every case.

Staphylococcus aureus is not only found in the great majority of furuncles, carbuncles, abscesses, and other inflammatory diseases of the surface of the body, but also plays an important rôle in a number of deeply seated diseases. Becker and others obtained it from the pus of osteomyelitis, demonstrating that if, after fracturing or crushing a bone, the staphylococcus be injected into the circulation, osteomyelitis may occur. Numerous observers have demonstrated its presence in ulcerative endocarditis. Rodet has been able to produce osteomyelitis without previous injury to the bones; Rosenbach was able to produce ulcerative endocarditis by injecting some of the staphylococci into the circulation in animals whose cardiac valves had been injured by a sound passed into the carotid artery; and Ribbert has shown that the injection of cultures of the organism may cause valvular lesions without preceding injury.

Virulence.—Experiments have shown that both Staphylococci aureus and albus exist in attenuated and virulent forms, and there is every reason to believe that in the majority of instances they inhabit the surface of the body in a feebly virulent condition.

Serum Therapy.—The treatment of streptococcus infections with immune serum has not met with encouraging success. Viquerat * has experimented in this direction and found that goats are best adapted to the manufacture of the serum; but the literature of medicine contains very little mention of "antistaphylococcus serum" and of beneficial results following its employment.

Staphylococcus Citreus (Passet).

An organism identical in many respects with the preceding, except that its growth on agar-agar and potato is of a brilliant lemon-yellow color, and its pathogenicity for animals doubtful, is *Staphylococcus citreus* of Passet.† As it is not common, and is doubtfully pathogenic, it is of much less importance than the previously described organisms.

Streptococcus Pyogenes (Rosenbach).

General Characteristics.—The streptococcus is a non-motile, non-flagellate, non-sporogenous, non-liquefying, aerobic and optionally anaerobic, spheric organism, infectious for man and the lower animals, whose division in one direction of space leads to its association in the form of chains or "strings of beads." It stains by ordinary methods and by Gram's method.

Streptococcus pyogenes (Fig. 76) was found by Rosenbach‡ in 18 of 33 cases of suppurative lesions, fifteen times by itself and five times in association with Staphylococcus aureus.

Morphology.—The organisms are spheric, of variable size (0.4–1 μ in diameter), and are constantly associated in pairs or in chains of from four to twenty or more individuals. Special varieties, known as Streptococcus longus (chains of more than one hundred members) and Streptococcus brevis

* "Zeitschrift für Hygiene," XVIII, 1894, p. 483.

† "Untersuchungen über die Aetiologie der eitrigen Phlegmone des Menschen," Berlin, 1885, p. 9.

‡ "Mikroorganismen bei Wundinfektionskrankheiten des Menschen," 1884, p. 22.

(chains of from four to ten), have been described by v. Lingelsheim.*

It is not motile and does not form endospores, though sometimes large individuals—much larger than the others in the chain—may be observed. Some believe these to be arthrospores.

Staining.—The organisms stain well with ordinary aqueous solutions of anilin dyes, and by Gram's method.

Isolation.—The streptococcus can be isolated from pus containing it, either by the usual method of "plating" or by the inoculation of a mouse or rabbit, from whose blood it may easily be secured after death.

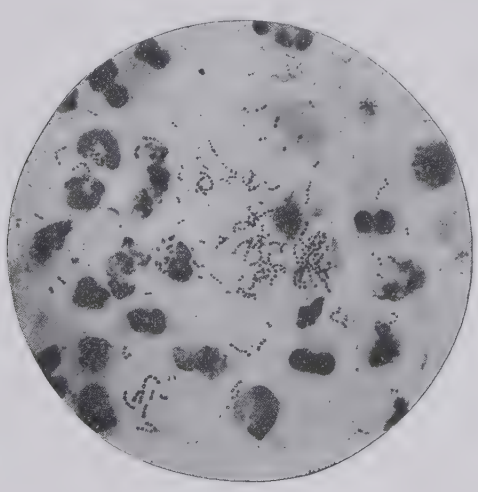


Fig. 76.—Streptococcus pyogenes, from the pus taken from an abscess. × 1000 (Fränkel and Pfeiffer).

Cultivation.—The organism grows at both the room temperature and that of incubation, its best and most rapid development being at about 37° C.

Colonies.—Upon gelatin plates very small, colorless, translucent colonies appear in from twenty-four to forty-eight hours. When superficial, they spread out to form flat disks about 0.5 mm. in diameter. The microscope shows them to be irregular and granular, to have a slightly yellowish color by transmitted light, and to have numerous irregularities

*"Zeitschrift für Hygiene," Bd. x, 1891, p. 331; xii, 1892, p. 308.

around the edges, due to projecting chains of the cocci. No liquefaction of the gelatin occurs.

Gelatin Punctures.—In gelatin puncture cultures no liquefaction is observed. The minute spheric colonies grow along the whole length of the puncture and form a slightly opaque granular line.

Agar-agar.—Upon agar-agar an exceedingly delicate transparent growth develops slowly along the line of inoculation. It consists of small, colorless, or slightly grayish transparent colonies which do not readily coalesce.

Blood-serum.—The growth upon blood-serum resembles that upon agar-agar. The colonies are small, white, discrete, and do not affect the medium.

Potato.—The streptococcus does not seem to grow well upon potato, the colonies being invisible.

Bouillon.—In bouillon the cocci develop slowly, seeming to prefer a neutral or feebly acid reaction. The medium remains clear, while numerous small flocculi are suspended in it, sometimes adhering to the sides of the tube, sometimes forming a sediment. When the flocculi-formation is distinct, the name *Streptococcus conglomeratus* (Kurth) is sometimes given to the organism; when the medium is diffusely clouded, it is called *Streptococcus diffusus*.

In mixtures of bouillon and blood-serum or ascitic fluid the streptococcus grows more luxuriantly, especially at incubation temperatures, distinctly clouding the liquid.

Milk.—The organism seems to grow well in milk, which is coagulated and digested.

Reaction.—The streptococcus is not very sensitive to acids, and can be grown quite well in media with a slightly acid reaction.

Vital Resistance.—Sternberg found that the streptococci succumb at temperatures of 52°–54° C. if maintained for ten minutes. Their vitality in culture is slight, and unless frequently transplanted they die. Bouillon cultures usually die in from five to ten days. On solid media they seem to retain their vegetative and pathogenic powers much longer, especially if kept cool and cultivated beneath the surface of the medium in a deep puncture. They resist drying well. The growth in artificial media is accompanied by the production of an acid which probably acts destructively upon the bacteria themselves and first inhibits further growth, then destroys them.

Pathogenesis.—The streptococcus has been found in erysipelas, ulcerative endocarditis, periostitis, otitis, meningitis, emphysema, pneumonia, lymphangitis, phlegmons, sepsis, puerperal endometritis, and many other forms of inflammation and septic infection. In man, it is usually associated with active forms of suppuration and sepsis.

The relation of the streptococcus to diphtheria is of interest, for, though in all probability the great majority of cases of pseudomembranous angina are caused by the Klebs-Löffler bacillus, yet a number are met with in which, as in Prudden's twenty-four cases, no diphtheria bacilli can be found, but which seem to be caused by the streptococcus.

There is no clinical difference between the throat-lesions produced by the two organisms, and the only positive method of differentiating the one from the other is by means of a careful bacteriologic examination. Such an examination should always be made, as it has much weight in connection with the treatment; in streptococcus angina no benefit can be expected from the administration of diphtheria antitoxic serum.

Hirsh* has shown that streptococci are by no means rare in the intestines of infants, where they may occasion enteritis. In such cases the organisms are found in large numbers in the stomach and in the stools, and late in the course of the disease in the blood and urine of the child. They also occur in all of the internal organs of the cadaver.

Libman† has reported two carefully studied cases of streptococcic enteritis.

Flexner,‡ in a large series of autopsies, found the bodies invaded by numerous micro-organisms, causing what he has called "terminal infections," and hastening the fatal issue. Of 793 autopsies at the Johns Hopkins Hospital, 255 upon cases dying of chronic heart or kidney diseases, or both, were sufficiently well studied, bacteriologically, to meet the requirements of a statistical inquiry. Tuberculous infections were not included. Of the 255 cases, 213 gave positive bacteriologic results. "The micro-organisms causing the infections, 38 in all, were Streptococcus pyogenes, 16 cases; Staphylococcus pyogenes aureus, 4 cases; Micrococcus lan-

* "Centralbl. f. Bakt. u. Parasitenk.," Bd. XXII, Nos. 14 and 15, p. 369.

† *Ibid.*, Bd. XXII, Nos. 14 and 15, p. 376.

‡ "Journal of Experimental Medicine," vol. I, No. 3, 1896.

ceolatus, 6 cases; gas bacillus (B. aerogenes capsulatus), three times alone and twice combined with Bacillus coli communis; the gonococcus, anthrax bacillus, Bacillus proteus, the last combined with Bacillus coli; Bacillus coli alone; a peculiar capsulated bacillus, and an unidentified coccus."

It is interesting to observe in how many cases the streptococcus was present. All the streptococci found may not have been Streptococcus pyogenes, but for convenience in his statistics they were regarded as such.

The presence of streptococci in the blood in scarlatina has been observed in 30 cases by Crooke, by Fränkel and Trendenburg, Raskin, Leubarth, Kurth, and Babes. In 11 cases of scarlatina studied by Wright * a general streptococcus infection occurred in 4, a pneumococcus infection in 1, and a mixed infection of pyogenic cocci in 1.

Lemoine † found streptococci in the blood during life in 2 out of 33 cases of scarlet fever studied. Pearce ‡ studied 17 cases of scarlatina and found streptococci in the heart's blood and liver in 4, in the spleen in 2, in the kidney in 5 cases. In 2 of the cases Staphylococcus pyogenes aureus was associated with the streptococcus.

The streptococcus is the most common organism found in the suppurative sequelæ of scarlatina, frequently occurring alone; sometimes with the staphylococci; sometimes with the pneumococci. Councilman found secondary infection by the streptococcus more widespread in variola than in any other disease.

Virulence.—Streptococcus pyogenes is very variable in its virulence for the lower animals, and seems to be most pathogenic for that species of animal from which it has been isolated. If isolated from man, it may not be virulent for mice, guinea-pigs, or rabbits, and *vice versâ*. If the cultures be of moderate virulence and the ear of a rabbit be carefully inoculated with a small quantity of a pure culture, local erysipelas usually results, the disturbance passing away in a few days and the animal recovering.

If, however, the streptococcus be highly virulent, the rabbit dies of general septicemia in from twenty-four hours to six days. The cocci may then be found in large numbers

* "Boston Med. and Surg Jour.," March 21, 1895.

† "Bull. et Mém. Soc. d'Hôp. de Paris," 1896, 3 s., XIII.

‡ "Jour. Boston Soc. of Med. Sci.," March, 1898.

in the heart's blood and in the organs. In less virulent cases minute disseminated *pyemic* abscesses are sometimes found.

According to Marmorek,* the virulence of the streptococcus can be increased to a remarkable degree by rapid passage through rabbits, and maintained by the use of a culture medium consisting of three parts of human blood-serum and one of bouillon. The blood of the ass or ascitic or pleuritic exudates may be used instead of the human blood-serum if the latter be unobtainable. By these means Marmorek succeeded in intensifying the virulence of a culture to such a degree that one hundred-thousand-millionth (*un cent milliardième*) of a cubic centimeter injected into the ear vein was fatal to a rabbit.

Petruschky † found the virulence of the culture to be well retained when the organisms were planted in gelatin, transplanted every five days, and when grown, kept on ice.

Holst ‡ observed a virulent Streptococcus brevis that remained unchanged upon artificial culture media for eight years without any particular precautions having been taken to maintain the virulence.

Dried streptococci are said by Frosch and Kolle § to retain their virulence longer than those growing on culture media.

Lubenau || found that cultures of the streptococcus when grown in glucose containing bouillon produced a hemolytic substance—streptokolysin—not seemingly present in cultures grown in ordinary bouillon. Besredka** found that streptokolysin was produced only by highly virulent cultures of the streptococcus and not by saprophytic organisms that have been for some time under cultivation in the laboratory.

Levin†† investigated the subject thoroughly and found that different strains of streptococci produced streptokolysin in very varying quantities, that its production is entirely independent of that virulence, that it is destroyed by heat (37° C. in some days; 55° C. in one-half hour); that acidity

* "Ann. de l'Inst. Pasteur," t. IX, No. 7, July 25, 1895, p. 593.

† "Centralbl. f. Bakt. u. Parasitenk.," Bd. XVIII, No. 16, May 4, 1895, p. 551.

‡ *Ibid.*, Bd. XIX, No 11, March 21, 1896.

§ Flügge's "Die Mikroorganismen."

|| "Centralbl. f. Bakt.," etc., 1901, Bd. XXX, Nos. 9 and 10.

** "Annales de l'Inst. Pasteur," 1901, S. 880.

†† "Nord. Med. Ark.," 1903, II, No. 15, p. 20.

of the nutrient media hinders its formation, and that it is intimately associated with the bodies of the streptococci by which it is produced, so that in the sediment obtained by filtration or by centrifugation there is nearly one thousand times as much as in the filtered fluid culture. The streptokolysin is not destroyed by the death of the bacteria. *Antistreptokolysin* is present in antistreptococcus serum.

Toxic Products.—The toxic products of the streptococcus are not well known. Cultures from different sources vary greatly in the effects produced by hypodermic or intravenous injection after filtration through porcelain. Killed cultures produce a much more marked effect than filtered ones.

In general the effects of streptococcus intoxication are vague. The animals appear weak and ill, and have a slight fever; but unless the virulence of the culture be exceptional or the dose very large, they usually recover in a short time.

The clinical observation that occasional accidental erysipelatous infection of malignant tumors is followed by sloughing and the subsequent disappearance of the tumor, suggested the experimental inoculation of such tumors with Streptococcus erysipelatis as a therapeutic measure. The danger of the remedy, however, caused many to refrain from its use, for when one inoculates the living erysipelas germs into the tissues it is impossible to estimate the exact amount of disturbance that will follow.

Coley's Mixture.—To overcome this difficulty Coley* has recommended that the toxin instead of the living coccus be used for injection. A virulent culture of the streptococcus is obtained, by preference from a fatal case of erysipelas, inoculated into small flasks of slightly acid bouillon, and allowed to grow for three weeks. The flask is then reinoculated with Bacillus prodigiosus, allowed to grow for ten or twelve days at the room temperature, well shaken up, poured into bottles of about f℥ss capacity, and rendered perfectly sterile by an exposure to a temperature of 50°–60° C. for an hour. It is claimed that the combined products of the streptococcus of erysipelas and Bacillus prodigiosus are much more active than a simple streptococcus culture. The best effects follow the treatment of cases of inoperable spindle-cell sarcoma, where the toxin

* "Amer. Jour. Med. Sci.," July, 1894.

sometimes causes a rapid necrosis of the tumor tissue, which can be scraped out with an appropriate instrument. Numerous cases are on record in which this treatment has been most efficacious; but, although Coley still recommends it and Czerny upholds it, the majority of surgeons have failed to secure the desired results.

Antistreptococcus Serum.—Since 1895 considerable attention has been bestowed upon the antistreptococcus serum of Marmorek * and Gromakowsky,† which is said to act specifically upon streptococcus infections, both general and local. Numerous cases of suppuration, septic infection, puerperal fever, and scarlatina are upon record in which the serum seems to have exerted a beneficial action, and it may be that antiphlogistic serums will occupy an important place in the medicine of the future.

The serum is prepared by the injection of cultures of living virulent streptococci into horses until a high degree of immunity is attained. The serum is probably both antitoxic and bacteriologic in action.

The success following the serums of some experimenters upon certain cases, and their failure and the universal failure of the serums of others, has suggested that there is considerable difference between different "strains" or families of streptococci. To obviate this Van de Velde has made a polyvalent antistreptococcus serum by using a number of different cultures secured from the most diverse clinical cases of streptococcus infection. Another of Tavel and Moser is made by using cultures from different cases of scarlatina. The use of these serums, however, has not given the satisfaction expected, and at the present moment the whole subject of antistreptococcus serums is debatable both from the standpoint of its theoretical scientific basis and its therapeutic application.

Streptococcus Erysipelatis (Fehleisen).

The streptococcus of Rosenbach is generally thought to be identical with a streptococcus described with Fehleisen ‡ as *Streptococcus erysipelatis* (Fig. 77).

The streptococcus of erysipelas can be obtained in almost

* "Ann. de l'Inst. Pasteur," t. IX, No. 7, July 25, 1895, p. 593.

† "Ann. de l'Inst. Pasteur," t. IX, No. 7, July 25, 1895.

‡ "Verhandlungen der Würzburger med. Gesellschaft," 1881.

pure culture from the serum which oozes from a puncture made in the margin of an erysipelatous patch. They are small cocci, usually forming chains of from six to ten individuals, but sometimes reaching a hundred or more in number. Occasionally the chains occur in tangled masses.

They can be cultivated at the room temperature, but grow much better at 30°–37° C. They are not particularly sensitive to the presence or absence of oxygen, but perhaps develop a little more rapidly in its presence. The cultural appearances are identical with those of Streptococcus pyogenes.

When injected into animals Fehleisen's coccus behaves exactly like Streptococcus pyogenes.

Many observations have shown that dire results follow the entrance of this organism into exposed wounds, where it sometimes causes local suppuration, sometimes general infection. The streptococci of erysipelas are usually highly virulent.

Bacillus Pyocyaneus (Gessard).

General Characteristics.—A minute, slender, actively motile, flagellated, non-sporogenous, chromogenic and feebly pathogenic, aerobic or facultative anaerobic, liquefying bacillus, staining by ordinary methods, but not by Gram's method.

In some cases pus has a peculiar bluish or greenish color, which depends upon the presence of *Bacillus pyocyaneus* (Figs. 78, 79) of Gessard.*

The bacillus appears to be a rather common saprophyte upon the skin and mucous membranes, and has been found in the perspiration.

Morphology.—It is a short, slender organism with rounded ends, measuring 0.3 × 1–2 μ, according to Flügge. It is frequently united in chains of four or six. It is actively motile, has one terminal flagellum, and does not form spores. It can exist without free oxygen, though it is an almost purely aerobic organism.

It closely resembles a harmless bacillus found in water and known as Bacillus fluorescens liquefaciens, from which Ruzicka† thinks it has probably descended.

Staining.—It stains well with the ordinary staining solutions, but not by Gram's method.

*"De la Pyocyanine et de son Microbe," Thèse de Paris, 1882.

†"Centralbl. f. Bakt. u. Parasitenk.," July 15, 1898, p. 11.

Fig. 77.—Streptococcus erysipelatis, seen in a section of human skin. × 500 (Fränkel and Pfeiffer).

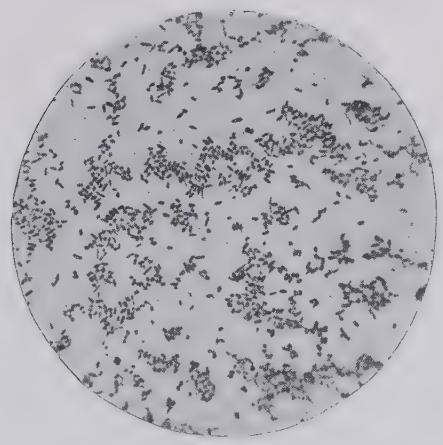


Fig. 78.—Bacillus pyocyaneus, from an agar-agar culture. × 1000 (Itzerott and Niemann).

Isolation.—The isolation of the organism is simple, the ordinary plate method being a satisfactory means of securing it from pus or other discharges containing it.

Cultivation.—Colonies.—The superficial colonies upon gelatin plates are small, irregular, slightly greenish, ill-defined, and produce a distinct fluorescence of the neighboring gelatin.

Microscopic examination shows the superficial colonies to be rounded, and coarsely granular with serrated or slightly filamentous borders. They are distinctly green in the center and pale at the edges. The colonies sink into the gelatin as

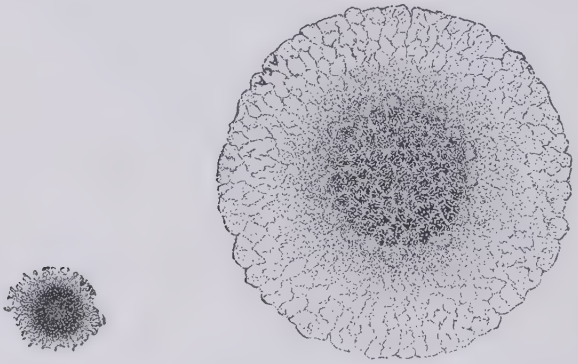


Fig. 79.—Bacillus pyocyaneus. Colonies upon gelatin (Abbott).

the liquefaction progresses. Four or five days must elapse before the medium is all fluid.

Gelatin Punctures.—In gelatin puncture cultures the chief development of the organisms occurs at the upper part of the tube, where a deep saucer-shaped liquefaction forms, slowly descending into the medium, and causing a beautiful fluorescence. At times a delicate scum forms on the surface, sinking to the bottom as the culture ages, and ultimately forming a slimy sediment.

Agar-agar.—Upon agar-agar the growth developing all along the line of inoculation at first appears bright green. The green color depends upon a soluble pigment (fluorescin) which soon saturates the culture medium and gives it the characteristic fluorescent appearance. As the culture ages, or if the medium upon which it grows contain much peptone, a second blue pigment (pyocyanin) develops, and the bright green fades to a deep blue-green, dark blue, or

in some cases to a deep reddish-brown color. This pigment has been made the subject of a careful investigation by Jordan.*

A well-known feature of the growth upon fresh agar-agar, upon which much stress has recently been laid by Martin,† is the formation of crystals in fresh cultures. Crystal-formation in cultures of other bacteria usually takes place in old, partially dried agar-agar cultures, but Bacillus pyocyaneus often produces crystals in a few days upon fresh media. In my experience freshly isolated bacilli show this power more markedly than those which have been for some time part of the laboratory stock of cultures and frequently transplanted.

Bouillon.—In bouillon the organism produces a diffuse cloudiness, a fluorescence, and sometimes an indefinite thin pellicle on the surface.

Potato.—Upon potato a luxuriant greenish or brownish, smeary layer is produced.

Milk.—Milk is coagulated and peptonized. It is slightly acid for the first day or two, then becomes alkaline again.

Pathogenesis.—The bacillus is pathogenic for the small laboratory animals, but different cultures differ greatly in virulence, 1 c.c. of a virulent bouillon culture, injected into the subcutaneous tissue of a guinea-pig or a rabbit, causing rapid edema, suppurative inflammation, and death in a short time (twenty-four hours). Sometimes the animal lives for a week or more, then dies. There is a marked hemorrhagic subcutaneous edema at the seat of inoculation. The bacilli can be found in the blood and in most of the tissues.

Doses too small to prove fatal sometimes lead to suppuration, and the injection of sterilized cultures leads to similar results, a relatively larger quantity being required.

Intraperitoneal injections cause purulent peritonitis.

Blum‡ reports a case of pyocyaneus infection with endocarditis in a child.

Lartigau,§ in his study of "The Bacillus Pyocyaneus as a Factor in Human Pathology," sums up what is known about this rôle of the organism as follows: "The Bacillus pyocyaneus, like many pathogenic micro-organisms, is

*"Journal of Experimental Medicine," vol. IV, 1899.

†"Centralbl. f. Bakt.," XXI, April 6, 1897, p. 473.

‡"Centralbl. f. Bakt. u. Parasitenk.," Feb. 10, 1899, XXV, No. 4.

§"Phila. Med. Jour.," Sept. 17, 1898.

occasionally found in a purely saprophytic rôle in various situations in the human economy. It has been found in the saliva by Pansini, in sputum by Frisch, and in the sweat by Eberth and Audanard. Abelous demonstrated its presence in the stomach as a saprophyte. Its existence in suppurating wounds has long been known, and Koch early detected its presence in tuberculous cavities, regarding it as an organism incapable of playing any pathologic rôle. The etiologic relation of the organism to certain cases of purulent otitis media in children was pointed out by Martha, Maggiora and Gradenigo, Babes, Kossel, and others. H. C. Ernst obtained it from a pericardial exudate during life. G. Blumer demonstrated its presence in practically pure cultures in a case of acute angina simulating diphtheria; Jadkewitsch, B. Motz, and Le Noir obtained the bacillus in cases of urinary infection. The cases of Triboulet, Karlinski, Oettinger, Ehlers, and Barker are interesting instances of its rôle in cutaneous lesions.

"In addition to these lesions, other morbid processes have been associated in some cases with the bacillus of blue pus, such as meningitis and oroncho-pneumonia by Monnier; diarrhea of infants by Neumann, Williams, Thiercelin and Lesage, and other observers; dysentery by Calmette and by Lartigau; and general infection by Ehlers, Neumann, Oettinger, Karlinski, Monnier, Krannhals, Calmette, Finkelstein, and L. F. Barker."

Nine additional cases of human infection are reported by Perkins.*

Immunity.—Immunity against pyocyaneus infection develops after a few inoculations with attenuated or sterilized cultures. These are easily prepared, the thermal death-point determined by Sternberg being 56° C.

Miscellaneous Organisms of Suppuration Described More Fully Elsewhere.

Before leaving the subject of suppuration, attention must be directed to other bacteria that under exceptional circumstances become the cause of suppuration. Among these are the pneumococcus of Fränkel and Weichselbaum, the typhoid bacillus, Bacillus coli communis, and the Micrococcus tetragenus. These organisms are considered under separate and appropriate headings, to which the reader is advised to refer.

* "Jour. of Med. Research," vol. VI, 1901, p. 281.

CHAPTER II.

GONORRHEA.

Micrococcus Gonorrhœæ (Neisser).

General Characteristics.—A minute, biscuit-shaped, non-motile, non-sporogenous, non-liquefying, non-flagellate, aerobic, strictly parasitic coccus, pathogenic for man only.

All authorities now accept the "gonococcus" as the specific cause of gonorrhea. It was first observed in the urethral and conjunctival secretions of gonorrhea and purulent ophthalmia by Neisser* in 1879.

Bumm † found other cocci closely resembling the gonococcus in the inflamed urethra, and points out that neither its shape nor its position in the cells can be regarded as characteristic, but that failure to stain by Gram's method can alone enable us to say with certainty that biscuit-shaped cocci found in urethral pus are gonococci.

Distribution.—The gonococcus is a purely parasitic pathogenic organism. It can be found in the urethral discharges of gonorrhea from the beginning until the end of the disease, and often for many months and even years after recovery from it. After the period of creamy pus has passed, its numbers are usually outweighed by other pyogenic organisms. Wertheim ‡ cultivated the gonococcus from a case of chronic urethritis of two years' standing, and proved its virulence by producing experimental gonorrhea in a human being. The organisms are chiefly found within the pus-cells (Fig. 80) or attached to the surface of epithelial cells, and should always be sought for as diagnostic of gonorrhea, as purulent urethritis is sometimes caused by other organisms, as Bacillus coli communis § and Staphylococcus pyogenes.

* "Centralbl. f. d. med. Wissenschaft," 1879, No. 28.

† "Der Mikroorganismus der gonorrhoischen Schleimhauterkrankungen," "Gonococcus Neisser," second edition, 1887.

‡ "Archiv f. Gynäkologie," Bd. xlii, 1892, Heft 1.

§ Van der Pluyn and Loag, "Centralbl. f. Bakt. u. Parasitenk.," Bd. xvii, Nos. 7, 8, Feb. 28, 1895, p. 233.

Morphology.—The organisms occur in pairs, the inner surfaces being flattened and separated from one another by a narrow interval. A good lens usually shows the approximated surfaces of the diplococci to be slightly concave. Sometimes, instead of diplococci, tetrads are seen, the group no doubt resulting from the division of a pair. A pair of the cocci resembles the German biscuit and is known to the Germans as *semmelförmig*.

The gonococci are small, not motile, not provided with flagella, and without spores.

Staining.—They stain readily with all the aqueous solu-

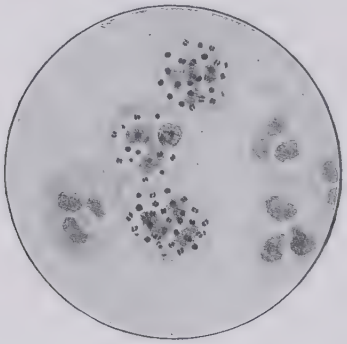


Fig. 80.—Gonococci in urethral pus.

tions of the anilin dyes—best with rather weak solutions, but not by Gram's method.

The organisms contained in pus can be beautifully shown by first treating the prepared film with alcoholic eosin, and then with Löffler's alkaline methylene-blue. A differential stain can be made by staining the film by Gram's method and then with aqueous Bismarck brown. Ordinary pus cocci, taking the Gram's stain, appear blue-black; the gonococci are dark brown.

Isolation and Cultivation.—The cultivation of the gonococcus is difficult and requires considerable bacteriologic skill.

The organism does not grow upon any of the ordinary culture media, and grows very scantily upon any artificial medium. Wertheim succeeded in cultivating it by diluting a drop of gonorrheal pus with a little liquid *human blood-serum*, mixing this with an equal part of melted 2 per cent.

agar-agar at 40° C., and pouring the mixture into Petri dishes, which, as soon as the medium became firm, were stood in the incubator at 37° C. In twenty-four hours the colonies could be observed. Those upon the surface showed a dark center, surrounded by a delicate granular zone.

Young * had excellent success with a hydrocele-agar prepared as follows: "The fluid (hydrocele or ascitic) is obtained sterile, the locality of the puncture being carefully sterilized by modern surgical methods, the sterile trocar covered at its external end with sterilized gauze so as not to be infected by the operator's hand, and the fluid collected in sterile flasks, the sterile stoppers being then replaced. Collecting the fluid in this way we have very rarely had it contaminated, often keeping it several months before using it. The fluid is mixed with ordinary nutrient agar. A number of common slants are put in the autoclave for five minutes. This liquefies the agar and at the same time thoroughly sterilizes the tubes and cotton stoppers. The slants are then put in a water-bath at 55° C. so as not to coagulate the albumin when mixed with the agar. The stopper having been removed from a small flask of hydrocele fluid, the top of the flask is flamed and the albuminous fluid is then poured into an agar tube (the top of which has also been flamed) in proportions a little more than one to two." The medium can be allowed to solidify in tubes or can be poured into Petri dishes.

When one of the colonies was transferred to a tube of human blood-serum, or of one of the above-described mixtures obliquely coagulated, isolated little gray colonies occur, later becoming confluent and producing a delicate smeary layer upon the medium. The main growth is surrounded by a thin, veil-like extension which gradually fades away at the edges. A slight growth also occurs in the water of condensation.

Turro † claims that gonococci may be cultivated upon acid gelatin, upon gelatin containing acid urine, and also in plain acid urine. The gonococci grow near the surface, while the pus cocci mixed with them sink deeper into the medium.

* "Contributions to the Science of Medicine by the Pupils of William M. Welch," Baltimore, 1900, p. 677.

† "Centralbl. f. Bakt. u. Parasitenk.," July 2, 1894, Bd. xvi, No. 1.

Heiman * found that the gonococcus grows best in a mixture of 1 part of pleuritic fluid and 2 parts of 2 per cent. agar. Wright † prefers a mixture of urine, blood-serum, peptone, and agar-agar.

Laitinen ‡ found agar-agar mixed with one-third to one-half its volume of cyst or ascitic fluid, and bouillon containing 1 per cent. of peptone and 0.5 per cent. of sodium chlorid, mixed with one-third to one-half its volume of cyst or ascitic fluid, very satisfactory. The gonococcus could be kept alive upon these media for two months. Laitinen found that the gonococcus produces acids in the early days of its development, and alkalies subsequently. He was unable to isolate any toxin from the cultures.

Vital Resistance.—The gonococci, though not easily cultivated, are said to resist unfavorable conditions, especially drying, very well. Kratter was able to demonstrate their presence upon washed clothing after six months, and found that they still stained well.

In artificial culture the gonococcus soon dies, though cultures from different sources differ considerably in this regard. As a rule they survive but a few transplantations, though Young found that one culture had been kept alive by students in his laboratory for more than three months.

Toxic Products.—The toxic metabolic products of the gonococcus appear to be contained within the bodies of the bacteria and disseminated but slightly throughout the culture media. Christmas, § Nicolaysen, ‖ and Wassermann ** have studied *gonotoxin*, and have all found that it remains in the bodies of the bacteria. The toxin seems to be quite stable and is not destroyed by temperatures fatal to the cocci. Wassermann obtained some cultures of which 0.1 c.c. would kill mice; others, of which 1.0 c.c. was required. The poison can be precipitated with absolute alcohol. Small quantities of the toxin introduced into the urethra cause suppuration at the point of application, fever,

* "Medical Record," Dec. 19, 1886.

† "Amer. Jour. Med. Sci.," Feb., 1895.

‡ "Centralbl. f. Bakt. u. Parasitenk.," June 1, 1898, vol. XII, No. 20, p. 874.

§ "Ann. de l'Inst. Pasteur," 1897.

‖ "Centralbl. f. Bakt. u. Parasitenk.," 1897, Bd. XXII, Nos. 12 and 13, p. 305.

** "Zeitschrift für Hygiene," 1898, and "Berliner klin. Wochenschrift," 1897, No. 32, p. 685.

swelling of the neighboring lymphatic nodes, and muscular and articular pains.

Pathogenesis.—It is generally believed that gonorrhea cannot be communicated to animals, but Turro (*loc. cit.*) asserts that when grown upon acid gelatin the gonococci readily communicate urethritis to dogs, and that no *læsio continui* is necessary, the simple introduction of the organisms into the *meatus urinarius* sufficing.

There is no doubt but that the gonococcus causes gonorrhea, as it has on several occasions been intentionally and experimentally inoculated into the human urethra with resulting typical disease. It is constantly present in the disease, and very frequently in its sequelæ, though it not infrequently happens that the lesions secondary to gonorrhea are caused by the more common organisms of suppuration that have entered through the surface denudations caused by the gonococcus.

The injection of gonococci into the subcutaneous tissue is not followed by either abscess-formation or septic infection.

Gonococci may enter the circulation of human beings and occasion a peculiar septic condition with irregular temperature, apt to be followed by invasion of the cardiac valves, joints or other tissues. P. Kraus* has twice succeeded in cultivating the gonococcus from the blood of patients in the stage of septic infection.

The deep lesions caused by the gonococcus are, however, numerous, and in Young's paper (*loc. cit.*) its widespread powers of pyogenic infection are well shown in a collection of the cases recorded in the literature, and some original observations showing the undoubted occurrence of the gonococcus in gonorrhea, ophthalmia neonatorum, arthritis, tendosynovitis, perichondritis, subcutaneous abscess, intramuscular abscess, salpingitis, pelvic peritonitis, adenitis, pleuritis, endocarditis, septicemia, acute cystitis, chronic cystitis, pyonephrosis, and diffuse peritonitis. In addition, Young observed the gonococcus in pyonephrosis, chronic cystitis, and diffuse peritonitis.

In the beginning of the inflammatory process the cocci grow in the superficial epithelial cells, but soon penetrate between the cells to the deeper layers, where they continue to keep up the irritation as the superficial cells desquamate. Opinions differ as to whether the gonococci can, with equal

* "Berl. klin. Woch.," No. 19, p. 494, May 9, 1904.

facility, penetrate squamous and columnar epithelium. Their attacks are usually made upon surfaces covered with squamous epithelium.

All urethral inflammations, and in gonorrhea all of the inflammatory symptoms, do not depend upon the gonococcus. The peri-urethral abscesses, salpingitis, etc., not infrequently depend upon ordinary pus cocci, and I remember having seen a case of gonorrhea with double orchitis, general septic infection, and endocarditis, in which the gonococci had no rôle in the sepsis, which was caused by a large dumb-bell coccus that stained beautifully by Gram's method.

In the remote secondary inflammations the gonococci disappear after a time, and the inflammation either subsides or is maintained by other bacteria. In synovitis, however, the inflammation excited may last for months.

So long as the gonococci persist in his urethra or other superficial tissue the patient may spread the contagion, and after apparent recovery from gonorrhea the cocci may remain latent in the urethra for years, not infrequently causing a relapse if the patient partake of some substance, as alcohol, irritating to the mucous membranes. Bearing this in mind, physicians should be careful that their patients are not too soon discharged as cured and permitted to marry.

Immunization against the gonococcus has not yet been successfully achieved by Wassermann, though Christmas claims to have immunized goats. The serum of these animals could not be shown to contain any antitoxin, and has not been shown to be bacteriolytic.

CHAPTER III.

CEREBRO-SPINAL MENINGITIS.

Diplococcus Intracellularis Meningitidis (Weichselbaum).

General Characteristics.—A minute non-motile, non-flagellate, non-sporogenous, non-liquefying, aerobic and optionally anaerobic, pathogenic coccus, staining by ordinary methods, but not by Gram's method.

Acute sero-purulent inflammation of the cerebral and spinal meninges frequently presents itself as a complication of well-known infectious processes, such as croupous pneumonia, and not infrequently occurs as a primary sporadic or epidemic affection. It is to the primary sporadic or epidemic cerebro-spinal meningitis that the following considerations are devoted. Cerebro-spinal meningitis is usually associated with one of three micro-organisms—the pneumococcus, the streptococcus, and Diplococcus intracellularis meningitidis of Weichselbaum. In more rare cases the staphylococci, the typhoid bacillus, and other bacteria may present themselves.

In the primary form of the disease Diplococcus intracellularis meningitidis seems to be the specific organism.

Distribution.—As early as 1887 Weichselbaum * carefully described a diplococcus found in six cases of cerebro-spinal meningitis that may have been identical with one found by Leichtenstern † in the purulent exudate of a case of meningitis. Weichselbaum's studies and description of this coccus seem to have attracted but little attention at first, and references to them are but brief in most of the text-books. The prevailing opinion as to its presence was that its occurrence in cerebro-spinal meningitis was accidental, as inoculations into animals showed its pathogenic power to be very limited. The careful studies of Jäger,‡ Scherer,§

* "Fortschritte der Med.," v, 18 and 19.

† "Deutsche med. Wochenschrift," 1885.

‡ "Zeitschrift für Hygiene," xix, 2, 351.

§ "Centralbl. f. Bakt. u. Parasitenk.," xvii, 13 and 14.

Councilman, and Mallory and Wright* (embracing fifty-five cases in which the cocci were found by culture or by microscopic examination in thirty-eight) have, however, shown the presence of the diplococcus of Weichselbaum in so large a number of cases that its importance has correspondingly increased.

The distribution of Diplococcus intracellularis in nature is as yet unknown. It has been found in cerebro-spinal meningitis by those who have looked for it, twice has been found in the nose in coryza by Scherer, has been found in the conjunctiva by Carl Fränkel † and Axenfeld,‡ and in the purulent discharges of rhinitis and otitis by Jäger.§ It occurs in above 50 per cent. of the cases of cerebro-spinal meningitis, but fails satisfactorily to fulfil the requirements of the laws of specificity.

Morphology.—The micro-organism is a biscuit-shaped diplococcus having a great resemblance to the gonococcus. This resemblance is further increased by the fact that the cocci are usually found inclosed in the protoplasm of the leukocytes. Weichselbaum, by whom this was first observed, found it constant in sections of the brain and its membranes, though in the exudate of the disease a good many free cocci may be observed. It was this peculiar relationship to the cells that led Weichselbaum to name the organism Diplococcus intracellularis. Many of the cocci inclosed in the cells are apparently dead and degenerated, as they stain badly and do not grow when the pus is transferred to culture media.

Carl Fränkel, in discussing the micro-organism, points out that its morphologic peculiarities have much in common with the pneumococcus, so that the most refined methods of differentiation should always precede a positive diagnosis. Its resemblance to the gonococcus should also be kept in mind.

Staining.—The organism is easily stained with the usual aqueous solutions of the anilin dyes. According to Weichselbaum, Mallory, and Wright, it does not stain by Gram's method.

* "Amer. Jour. Med. Sci.," March, 1898, vol. cxv, No. 5.

† "Zeitschrift für Hygiene," June 14, 1899.

‡ Lubarsch and Oestertag, "Ergebnisse der allg. Path. u. path. Anat.," III, S. 573.

§ "Deutsche med. Wochenschrift," 1894, S. 407.

For staining the meningococcus the method of Pick and Jacobsohn * is highly praised by Carl Fränkel, who modifies it by adding three times as much carbol-fuchsin as is recommended in the original instructions, which are as follows: Mix 20 c.c. of water with 8 drops of saturated methylene-blue solution; then add 45–50 drops of carbol-fuchsin. Allow the fluid to act upon the cover-glass for five minutes. The cocci alone are blue, all else red.

Isolation.—The organism can be secured for cultivation either from the purulent matter of the exudate found at autopsy, or from the fluid obtained by lumbar puncture. To obtain this fluid Park † gives the following directions: "The patient should lie on the right side with the knees drawn up and the left shoulder depressed. The skin of the patient's back, the hands of the operator, and the large antitoxin syringe should be sterile. The needle should be 4 cm. in length, with a diameter of 1 mm. for children, and larger for adults. The puncture is generally made between the third and fourth lumbar vertebræ. The thumb of the left hand is pressed between the spinous processes, and the point of the needle is entered about 1 cm. to the right of the median line and on a level with the thumb-nail, and directed slightly upward and inward toward the median line. At a depth of 3 or 4 cm. in children and 7 or 8 cm. in adults the needle enters the subarachnoid space, and the fluids flow out in drops or in a stream. If the needle meets a bony obstruction, withdraw and thrust again rather than make lateral movements. Any blood obscures microscopic examination. The fluid is allowed to drop into sterile test-tubes or vials with sterile stoppers. From 5 to 15 c.c. should be withdrawn. No ill effects have been observed from the operation."

In making a culture from this fluid Park points out that, as many of its contained cocci are dead, a considerable quantity of the fluid (say about 1 c.c.) must be used.

The cocci have also been cultivated from the nasal discharges in the six cases studied by Weichselbaum and in eighteen studied by Scherer. To determine the presence of the coccus in the nasal discharges where other similar cocci may be present, Gram's stain may be used and followed by

* "Berliner klin. Wochenschrift," 1896, S. 811.

† "Bacteriology in Medicine and Surgery," Philadelphia, 1899, p. 520.

an aqueous solution of Bismarck brown. The meningococci will be brown.

Cultivation.—The organism was successfully cultivated by Weichselbaum, but does not readily adapt itself to artificial media. It develops upon agar-agar and glycerin agar-agar, upon Löffler's blood-serum mixture, and, according to Goldschmidt,* upon potato. Weichselbaum did not find that it developed upon potato. It does not grow in bouillon or gelatin. There is nothing characteristic about the cultures. The cocci grow only at the temperature of the body, attain only a sparse development, and form a more or less confluent line of minute, rounded, grayish colonies which are easily overlooked upon opaque media like blood-serum. The general characteristics of the growth are not unlike those of the pneumococcus, streptococcus, and gonococcus.

Colonies.—When grown upon agar-agar plates, the deep colonies scarcely develop at all, appearing under the low-power lens as minute, irregularly rounded granular masses. The surface colonies are larger, and consist of an opaque yellowish-brown nucleus about which a flat, rounded disk spreads out. The edges may be dentate; the color is grayish or yellowish near the center, becoming less intense as the thin edges are reached; the structure is finely granular.

Vital Resistance.—The vitality of the culture is low, and the cocci die out readily, ceasing to grow when transplanted after eight or ten days. It becomes necessary, therefore, when studying the organism to transplant it frequently—Park † says every two days.

Pathogenesis.—The results of animal inoculations made with Diplococcus intracellularis meningitidis are disappointing. Subcutaneous inoculations into the lower animals are continually without effect. Intrapleural and intraperitoneal injections of cultures of the organism into mice and guinea-pigs are sometimes fatal, the dead animals showing a sero-fibrinous inflammation with the presence of the cocci. The intravenous injection of the coccus into rabbits is followed by death without important or conclusive symptoms, and usually without the presence of cocci in the blood.

Weichselbaum endeavored to reproduce the original cerebro-spinal meningitis in animals by trephining and in-

* "Centralbl. f. Bakt. u. Parasitenk.," II, 22, 23.

† "Bacteriology in Medicine and Surgery," 1899, p. 518.

jecting the cocci beneath the dura. In this manner he inoculated three rabbits and three dogs. Two of the rabbit injections failed, probably because the injected material escaped at once from the wound. The third rabbit died, and showed marked congestion of the membranes of the brain and a minute softened and hemorrhagic area. In these the cocci were found by culture to be abundant. The three dogs all died with congestion and pus-formation in the membranes and areas of softening in the brain substance. The cocci were recovered from two of the dogs, but the lesions of the third animal, which lived twelve days, contained none.

It is not known by what channels infection with Diplococcus intracellularis meningitidis takes place. Weichselbaum supposed it might enter by the nasal, auditory, or other passages, especially the nose, where he constantly found it. In this connection it is interesting to note that the only two of fifty supposedly healthy persons studied by Scherer in whom this coccus was found suffered from coryza, which is an almost constant early symptom of cerebro-spinal meningitis.

Steel* has found what may be a variety of the meningococcus in the simple posterior basic meningitis of infants. The organism differs from that of Weichselbaum in having a more permanent saprophytic existence upon culture media, where it often lives as long as thirty days. It is easily stained by methylene-blue, but not by Gram's method.

* "Pediatrics," Nov. 15, 1898.

CHAPTER IV.

CATARRHAL INFLAMMATION.

Micrococcus Catarrhalis (Seifert).*

General Charateristics.—A small, slightly ovoid, non-motile, non-sporulating, non-flagellated, non-liquefying aerobic and optionally anaerobic, non-chromogenic coccus, pathogenic for man, and not for the lower animals, cultivable upon the ordinary media, staining by the ordinary methods but not by Gram's method.

This micro-organism, which seems to be closely related to the staphylococci, was first observed, in sections of the lung of a case of influenza, by Seifert. It was successfully cultivated in 1890 by Kirchner† from ten cases of an influenza-like affection. It has since been frequently demonstrated in the exudates from various inflammatory conditions of the respiratory tract and conjunctiva, and seems to be a not uncommon organism of superficial inflammations. It is a rather troublesome organism, causing some confusion because of its disposition to occur in pairs, which gives it a close resemblance to the pneumococcus except in cases in which the capsules of the latter are very distinct. It is also readily taken up by the leukocytes, and may so resemble the gonococcus; and it is not always easy, perhaps not always possible, to distinguish it from the Diplococcus intracellularis meningitidis.

Morphology.—The organism is spherical or slightly ovoid, may occur singly, though usually appears in pairs or clusters. Large numbers are enclosed in the leukocytes or other cells. The spherical organisms have a diameter of about 1 μ; the ovoid organisms may measure as much as 1.5 by 2 μ. The relation of the cocci to the cells seems to have something to do with the course of the inflammatory conditions with which they are associated. During the activity of the process large numbers of the cells may be free; toward its close they may all be enclosed in the leukocytes.

*"Volkmann's klin. Vortr.," Nr. 240.

†"Zeitschr. f. Hyg.," Bd. 9.

The organisms are not motile and they have no flagella.

Staining.—The cocci stain by ordinary methods but not by Gram's method.

Cultivation.—The organism can be easily cultivated, the colonies being quite characteristic. They are large, white, irregular in outline, elevated at the center, not viscid, and grow readily at room temperatures upon all the culture media, the best upon blood-agar-agar. The vitality of the organism in culture is not great. Very often transplantations made after from four to six days fail to grow; and in

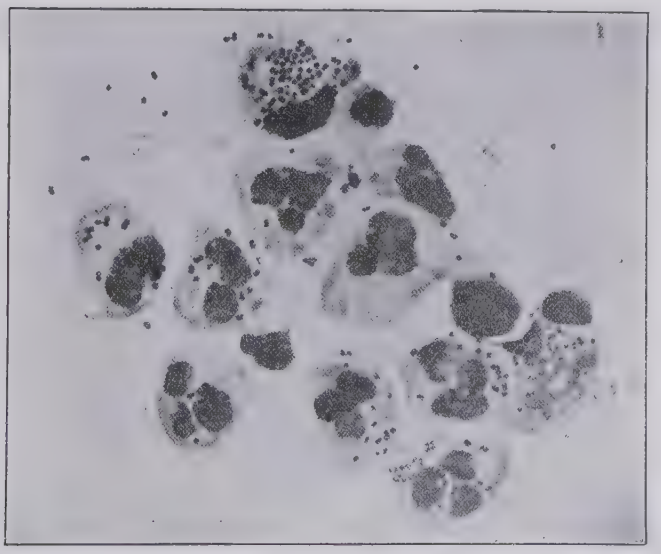


Fig. 81.—Micrococcus catarrhalis in smear from sputum (F. T. Lord; photo by L. S. Brown).

the cultures one usually finds many deeply-staining, supposedly living cocci, and many poorly-staining, supposedly dead organisms.

Agar-agar.—The culture in general resembles that of Staphylococcus albus. When blood is added to the agar-agar, the growth is more luxuriant, whitish, and usually consists of closely approximated colonies which do not become confluent.

Gelatin.—This medium is not liquefied.

Bouillon.—At the end of the first day no growth seems

to have taken place, but at the end of the second day there is a slight clouding and a meager precipitate. The organism seems to maintain its vitality somewhat longer in bouillon than in other culture media.

Pathogenesis.—The organism seems to be scarcely pathogenic for animals. Kirchner was able to kill a guinea-pig by intrapleural injection, and Neisser, who performed numerous experiments upon mice, guinea-pigs, and rabbits,

Fig. 82.—Micrococcus catarrhalis colonies on agar (F. T. Lord; photo by L. S. Brown).

only once succeeded in producing a fatal infection, by the intraperitoneal injection of 0.4 c.c. of bouillon culture. In this animal the cocci were found in all the internal organs. As has already been said, the organism is found associated with superficial inflammatory conditions of the mucous membrane. It is probably most common in influenza. It has also been found in conjunctivitis, in bronchitis, in whooping-cough, and in pneumonia.

CHAPTER V.

PNEUMONIA.

LOBAR OR CROUPOUS PNEUMONIA.

Diplococcus Pneumoniæ (Weichselbaum).

General Characteristics.—A minute, spheric, slightly elongate or lancet-shaped, non-motile, non-flagellate, non-sporogenous, aerobic and optionally anaerobic, non-chromogenic, non-liquefying diplococcus, pathogenic for man and the lower animals, staining by ordinary methods and by Gram's method.

"Pneumonia," while generally understood to refer to the lobar form of the disease particularly designated as *croupous pneumonia*, is a vague term, comprehending a number of quite dissimilar inflammatory conditions of the lung. This being true, no single micro-organism can be "specific" for all. Indeed, pneumonia must be conceived of as a group of diseases, and the various micro-organisms associated with it must be separately considered in connection with the particular varieties of the disease in which they occur.

The micro-organism, that can be demonstrated in at least 75 per cent. of cases of lobar pneumonia, which is almost universally accepted to be the cause of the disease, and about whose specificity very few doubts can now be raised, is the Diplococcus lanceolatus, or *pneumococcus* of Fränkel and Weichselbaum.

Priority of discovery of the pneumococcus seems to be in favor of Sternberg,* who as early as 1880 described an apparently identical organism which he secured from his own saliva. Pasteur † seems to have cultivated the same micro-organism, also from saliva, in the same year. The researches of the observers whose names are now attached to the organism were not completed until five years later. It is to Telamon,‡ Fränkel,§ and particularly to Weichsel-

* "National Board of Health Bulletin," 1881, vol. II.

† "Compte-rendus Acad. des Sciences," 1881, XCII, p. 159.

‡ Communication to the Société anatom. de Paris, Nov. 30, 1883.

§ "Deutsche med. Wochenschrift," 1885, 31.

baum,* however, that we are indebted for the discovery of the relation which the organism bears to pneumonia.

Distribution.—The pneumococcus is one of a group of widely disseminated organisms of the respiratory tract. It is characterized by certain peculiarities of morphology, certain metabolic peculiarities, a definite pathogenesis, and a distinct agglutinative reaction with immune serum. From such typical individuals a number of irregular departures are known, and recent researches make it certain that

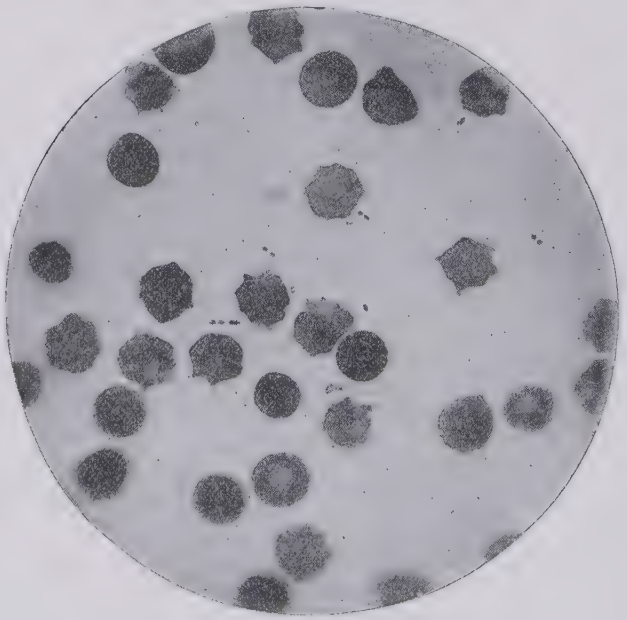


Fig. 83.—Diplococcus pneumoniæ, from the heart's blood of a rabbit. × 1000 (Fränkel and Pfeiffer).

many of the organisms formerly looked upon as pneumococci are different and perhaps harmless. The pneumococcus is a purely parasitic, pathogenic organism, best known to us in its relation to croupous pneumonia, where it is present in the lungs, sputum, and blood. It may be found in the saliva of a large number of healthy persons (Parke and Williams†), especially during the winter months (Longcope

*"Wiener med. Jahrbuch," 1886, p. 483.

†"Jour. Exp. Med.," VII, Aug. 7, 1905, p. 403.

and Fox*), and the inoculation of human saliva into rabbits frequently causes septicemia in which the pneumococci are abundant in the blood and tissues. Its frequent occurrence in the saliva led Flügge to describe it as Bacillus septicus sputigenus. It is occasionally found in inflammatory lesions other than pneumonia, as will be pointed out below.

Morphology.—The organism (Fig. 83) is variable in morphology. When grown in bouillon it appears oval, has a pronounced disposition to occur in pairs, and not infrequently forms chains of five or six members, so that some have been disposed to look upon it as a streptococcus (Gamalëia). In the fibrinous exudate from croupous pneumonia, in the rusty sputum, and in the blood of rabbits and mice containing them, the organisms occur in pairs, have a lanceolate shape, the pointed ends usually being approximated, and are usually surrounded by a distinct halo or capsule of clear, colorless, homogeneous material, thought by some to be a swollen cell-wall, by others a mucus-like secretion given off by the cells. When grown in culture media, especially upon solid media, the capsules are not apparent. This elongate form has led Migula† to describe it under the name Bacterium pneumoniæ.

The organism is without motility, has no flagella, forms no spores, and seems unable long to resist unfavorable conditions when grown artificially.

Staining.—It stains well with the ordinary solutions of the anilin dyes, and gives most beautiful pictures in blood and tissues when stained by Gram's and Weigert's methods.

To demonstrate the capsules, the glacial acetic acid method of Welch‡ may be used. The cover-glass is spread with a thin film of the material to be examined, which is dried and fixed as usual. Glacial acetic acid is dropped upon it for an instant, poured (not washed) off, and at once followed by anilin-water gentian violet, in which the staining continues several minutes, the stain being poured off and replaced several times until the acid has all been removed.

Finally, the preparation is washed in water containing 1 or 2 per cent. of sodium chlorid, and may be examined at once in the salt solution, or mounted in balsam after drying.

*"Jour. Exp. Med.," VII, Aug. 7, 1905, p. 430.

†"System der Bakterien," Jena, 1900, p. 347.

‡"Bull. of the Johns Hopkins Hospital," Dec., 1892, p. 128.

The capsules are more distinct when the examination is made in water.

Hiss* recommends the following as an excellent method of staining the capsules of the pneumococcus: The organism is first cultivated upon ascites-serum-agar to which 1 per cent. of glucose is added. The drop containing the bacteria to be stained is spread upon a cover-glass mixed with a drop of serum or a drop of the fluid culture medium, and dried and fixed. A half-saturated aqueous solution of gentian violet is applied for a few seconds and then washed

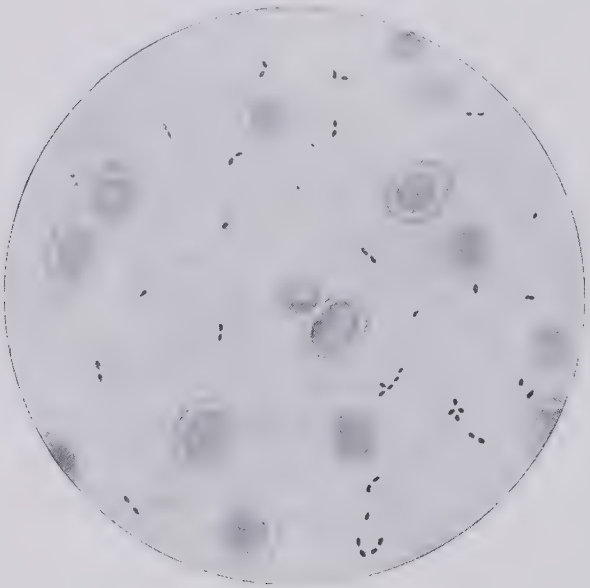


Fig. 84.—Capsulated pneumococci in blood from the heart of a rabbit; carbol-fuchsin, partly decolorized. × 1000.

off in a 25 per cent. solution of carbonate of magnesium. The preparation is then mounted in a drop of the latter solution and examined.

If it is desired to stain the capsules and preserve the specimens permanently in balsam, Hiss employs a 5 or 10 per cent. solution of fuchsin or gentian violet (5 c.c. saturated

*Abstract, "Centralbl. f. Bakt. u. Parasitenk.," Bd. xxxi, No. 10, p. 302, March 24, 1902.

alcoholic solution of dye in 95 c.c. of distilled water). The stain is applied to the fixed specimen and heated until it begins to steam, when the stain is washed off in a 20 per cent. solution of crystals of sulphate of copper. The preparation is then dried and mounted in balsam.

Hiss finds this stain a useful aid in differentiating the pneumococcus from the streptococcus with which it is easily confounded if the capsules are not distinct, and to which it is probably closely related.

Isolation.—When desired for purposes of study, the pneumococcus may be obtained by inoculating rabbits with pneumonic sputum and recovering the organisms from the heart's blood, or it may be secured from the rusty sputum of pneumonia by the method employed by Kitasato for securing tubercle bacilli from sputum: A mouthful of fresh sputum is secured, washed in several changes of sterile water to free it from the bacteria of the mouth and pharynx, carefully separated, and a minute portion from the center transferred to an appropriate culture medium.

Buerger,* in conducting a research upon pneumococcus and allied organisms with reference to their occurrence in the human mouth, under the auspices of the Rockefeller Institute, used a 2 per cent. glucose-agar of a *neutral*, or, at most, 0.5 per cent. phenolphthalein acid titre.

"The medium was usually made from meat infusion and contained 1.5 to 2 per cent. peptone and 2.5 per cent. agar. Stock plates of these media (serum-agar and 2 per cent. glucose-serum-agar) were poured. The agar or glucose-agar was melted in large tubes and allowed to cool down to a temperature below the coagulation point of the serum. One-third volume of rich albuminous ascitic fluid was added, and the resulting media poured into Petri-plates. These were tested by incubation and stored in the ice-chest ready for use. * *

"The plan finally adopted [for inoculating the plates] was as follows: A swab taken from the mouth was thoroughly shaken in a tube of neutral bouillon. From this primary tube dilutions in bouillon with four, six and eight loops may be made. A small portion of the dilute mixture was poured at a point near the periphery of the prepared plates. By a slight tilting motion the fluid was carefully distributed over the whole surface of the plates. Care must be taken to avoid an excess of fluid. It was found that plates made in this way gave a sufficiently thick and discrete distribution of surface colonies.

Cultivation.—The organism grows upon all the culture media except potato, but only between the temperature extremes of 24° and 42° C., the best development being at about 37° C. The growth is always meager, probably be-

* "Jour. Exp. Med.," Aug. 25, 1905, VII, No. 5.

cause of the metabolic formation of formic acid. The addition of alkali to the culture medium favors the growth of the pneumococcus by neutralizing this acid.

Colonies.—The colonies which develop at 24° C. upon gelatin plates (15 per cent. of gelatin should be used to prevent melting at the temperature required) are described as small, round, circumscribed, finely granular white points which grow slowly, never attain any considerable size, and do not liquefy the gelatin.

If agar-agar be used instead of gelatin, and the plates kept at the temperature of the body, the colonies appear transparent, delicate, and dewdrop-like, scarcely visible to the naked eye, but under the microscope appearing distinctly granular, the dark center being surrounded by a paler marginal zone.

Upon the medium recommended for isolating the pneumococcus by Buerger, the colonies appear in from eighteen to twenty-four hours, the surface colonies appearing circular and disc-like. When viewed from above, the surface appears glassy with a depressed center. When viewed from the side or by transmitted light, they appear as distinct milky rings with a transparent center. This "ring type" is regarded as characteristic and enables the organism to be separated without difficulty from the streptococcus.

Gelatin Punctures.—In gelatin puncture cultures, made with 15 instead of the usual 10 per cent. of gelatin, the growth takes place along the entire puncture in the form of minute whitish granules distinctly separated from one another. The growth in gelatin is always very meager.

Agar-agar and Blood-serum.—Upon agar-agar and blood-serum the growth consists of minute, transparent, semi-confluent, colorless, dew-drop-like colonies, which die before attaining a size which permits of their being seen without careful inspection. Upon glycerin agar-agar the growth is more luxuriant. The addition of a very small percentage of blood-serum greatly facilitates the growth.

Bouillon.—In bouillon the organisms grow well, slightly clouding the medium.

Milk.—Milk is an appropriate culture medium, its casein being coagulated. Alkaline litmus milk is slowly acidified.

Potato.—The pneumococcus does not grow upon potato.*

* Ortmann asserts that the pneumococcus can be grown on potato at 37° C., but this is not generally confirmed. The usual acid reaction of the potato would indicate that it was a very unsuitable culture medium.

Vital Resistance.—Bordoni-Uffreduzzi found that when pneumococci were dried in sputum attached to clothing, and were exposed freely to the light and air, they retained their virulence for rabbits for from nineteen to ninety-five days. Direct sunlight destroyed their virulence in twelve hours. Guarniere found that dried blood containing pneumococci remained virulent for months.

Metabolic Products.—Hiss* found that the pneumococcus produces acid with ease from monosaccharids, disaccharids, and such complex saccharids as dextrin, glycogen, starch, and inulin.

Toxic Products.—Nothing definite is known about the metabolic toxic products of the pneumococcus. That the symptoms of pneumonia are not entirely dependent upon the disturbance of respiration is clearly shown by the fact that the patients suffer from high fever and have marked leukocytosis with enlargement of the spleen. The cases in which the cocci invade the blood are usually more serious than those in which their operations are restricted to the lung.

The toxin must be purely or almost purely intracellular, however, as filtered cultures are scarcely at all toxic.

Auld † found that if a thin layer of prepared chalk were placed upon the bottom of the culture-glass, it neutralized the lactic acid produced by the pneumococcus, and enabled it to grow better and produce much stronger toxin.

Pathogenesis.—If a small quantity of a pure culture of the virulent organism be introduced into a mouse, rabbit, or guinea-pig, the animal dies in one or two days. Exactly the same result can be obtained by the introduction of a piece of the lung-tissue from croupous pneumonia, by the introduction of some of the rusty sputum, and frequently by the introduction of human saliva. Post-mortem examination of infected animals shows an inflammatory change at the point of subcutaneous inoculation, with a fibrinous exudate similar to that succeeding subcutaneous inoculation with the diphtheria bacillus. At times, and especially in dogs, a little pus may be found. The spleen is enlarged, firm, and red-brown. The blood with which the cavities of the heart are filled is firmly coagulated and, like that in other organs of the body, contains large numbers

* "Jour. Exp., Med.," VII, No. 5, Aug. 25, 1905.

† "Brit. Med. Jour.," Jan. 20, 1900.

of the bacteria, most of which exhibit a lanceolate form and have distinct capsules. The disease is thus shown to be a bacteremia unassociated with conspicuous tissue-changes.

In such cases the lungs show no consolidation. Even if the inoculation be made by a hypodermic needle plunged through the breast-wall into the pulmonary tissue, pneumonia rarely results. Monti claims to have found that a characteristic croupous pneumonia results from the injec-

Fig. 85.—Lung of a child, showing the appearance of the organ in the stage of red hepatization of croupous pneumonia. The pneumonia has been preceded by chronic pleuritis, which accounts for the thickened fibrous trabeculæ extending into the tissue, and which may have had something to do with the peculiarly prominent appearance of the bronchioles throughout the lung.

tion of cultures into the trachea of susceptible animals. This observation, however, lacks confirmation.

Lesions.—The lesions of croupous pneumonia of man are almost too well known to need description. The distribution of the disease conforms more or less perfectly to the divisions of the lung into lobes, one or more lobes being affected. An entire lung may be affected, though as a rule the apex escapes consolidation and is simply congested. The invaded portion of the lung is supposed to pass through a succession of stages clinically described as (1) congestion, (2) red hepatization, (3) gray hepatization, and (4) resolution. In the first stage bloody serum is poured out into the air-cells, filling them with a viscid reddish exudate. In the second stage this coagulates so that the tissue becomes solid, airless, and approximately like liver-tissue in appearance. The third stage is characterized by dissolution of the erythrocytes and invasion of the diseased air-cells by leukocytes, so that the color of the tissue changes from red to gray. At the same time the coagulated exudate begins to soften and leave the air-cells by the natural passages, and the stage of resolution begins.

In more rare cases circumscribed areas of consolidation occur in the lung-tissue. The inflammatory lesions of other organs present nothing characteristic by which they can be recognized by macroscopic examination.

The pneumococcus is not infrequently discovered in diseased conditions other than croupous pneumonia; thus, Foa, Bordoni-Uffreduzzi, and others found it in cerebro-spinal meningitis; Fränkel, in pleuritis; Weichselbaum, in peritonitis; Banti, in pericarditis; numerous observers in acute abscesses; Gabbi isolated it from a case of suppurative tonsillitis; Axenfeld observed an epidemic of conjunctivitis caused by it; Zaufal, Levy, and Schröder and Netter have been able to demonstrate it in the pus of otitis media, and Foulerton and Bonney* isolated it from a case of primary infection of the puerperal uterus. It has also been found in arthritis following pneumonia, and in primary arthritis without previous pneumonia by Howard.†

Interesting statistics concerning the relative frequency of pneumococcus infections in adults given by Netter‡ are as follows:

* "Trans. Obstet. Soc. of Lond.," 1903, part II, p. 128.

† "Johns Hopkins Hospital Bulletin," Nov., 1903.

‡ "Compte-rendu," 1889.

Pneumonia	65.95
Broncho-pneumonia	15.85
Meningitis	13.00
Empyema	8.53
Otitis media	2.44
Endocarditis	1.22
Hepatic abscess	1.22

In 46 consecutive pneumococcus infections of children he found:

Otitis media	29
Broncho-pneumonia	12
Meningitis	2
Pneumonia	1
Pleurisy	1
Pericarditis	1

Susceptibility.—Not all animals are susceptible to the action of the pneumococcus. Guinea-pigs, mice, and rabbits are highly sensitive, dogs comparatively immune.

Specificity.—The etiologic relationship of the pneumococcus to pneumonia is based more upon the frequency with which it is found in that disease than upon its ability to produce a similar affection in the lower animals, and we are still unable to furnish an absolute proof of specificity according to the postulates of Koch. The invariability of its presence in croupous pneumonia is, however, very convincing, as Netter * found it 82 times in 82 autopsies upon such cases; Klemperer, 21 times out of 21 cases studied by puncturing the lung with a hypodermic syringe. Weichselbaum obtained it in 94 out of 129 cases; Wolf, in 66 out of 70; and Pierce, in 110 out of 121 cases. In about 5 per cent. of the cases it remains localized in the respiratory apparatus; in 95 per cent. it invades the blood. An interesting paper upon this subject has been written by E. C. Rosenow.†

The conditions under which it enters the lung to produce pneumonia are not known. It is probable that some systemic depravity is necessary to establish susceptibility, and in support of this view we may point out that pneumonia is very frequent, and exceptionally severe and fatal, among drunkards, and that it is the most frequent cause of death among the aged. Whether, however, any particular form of vital depression is necessary to predispose to the disease, further study will be required to tell.

* "Compte-rendu," 1889.

† "Jour. Infectious Diseases," 1904, 1, p. 280.

Virulence.—Pneumococci vary greatly in virulence, and rapidly lose this quality in artificial culture. When it is desired to maintain or increase the virulence, a culture must be frequently passed through animals. Washbourn found, however, that a pneumococcus isolated from pneumonic sputum and passed through one mouse and nine rabbits developed a permanent virulence when kept on agar-agar so made that it was not heated beyond 100° C., and alkalinized 4 c.c. of normal caustic soda solution to each liter beyond the neutral point determined with rosolic acid. The agar-agar is first streaked with sterile rabbit's blood, then inoculated. The cultures are kept at 37.5° C. Ordinarily pneumococci seem unable to accommodate themselves to a purely saprophytic life, and unless continually transplanted to new media die in a week or two, sometimes sooner. Lambert found, however, that in Marmorek's

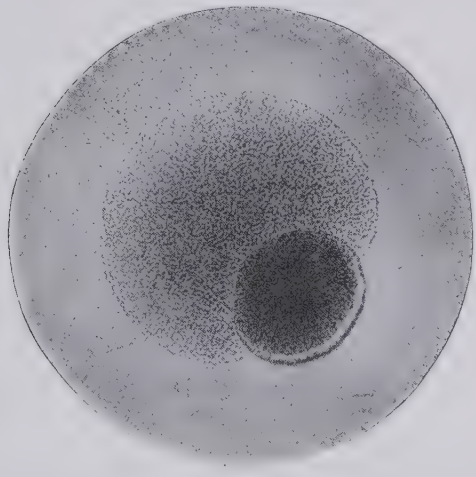


Fig. 86.—Diplococcus pneumoniæ. Colony twenty-four hours old upon gelatin. × 100 (Fränkel and Pfeiffer).

mixture of bouillon 2 parts and ascitic or pleuritic fluid 1 part, the organisms would sometimes remain alive as long as eight months, preserving their virulence during the entire time.

Virulence can also be retained for a considerable time by keeping the organisms in the blood from an infected rabbit, hermetically sealed in a glass tube, on ice.

Bacteriologic Diagnosis.—It is usually unnecessary to call upon the bacteriologist to assist in making the diagnosis of pneumonia. If necessary, the expectoration can be examined by the methods already given for staining the pneumococcus, or rabbits may be inoculated and the organism recovered from the blood. Caution must be exercised in using this means of diagnosis, however, as the organism sometimes occurs in normal saliva, and is a common associated organism in tuberculosis and other respiratory diseases. Wadsworth* has been able to show that agglutination reactions can be obtained by concentrating the pneumococci in isotonic solution and adding the serum. The method does not seem easily applicable for diagnosis.

Buerger† found that all pneumococci irrespective of source were agglutinated by pneumococcus immune serum, that such serum was capable of agglutinating various pyogenic streptococci, certain atypical organisms and certain strains of Streptococcus mucosus capsulatus. The sera of pneumonia patients varies in its power to agglutinate different pneumococci; some strains were agglutinated, others not. The sera of normal individuals and of normal rabbits possess no agglutinating power for pneumococci, the atypical organisms, certain streptococci and the Streptococcus mucosus capsulatus.

Immunity.—Pneumonia is peculiar in that recovery is followed by immunity of such brief duration as to permit the occurrence of frequent relapses; and it is well known that many cases show a subsequent predisposition to fresh attacks of the disease.

Immune Serum.—G. and F. Klemperer‡ have shown that the serum of rabbits immunized against the pneumococcus protects animals infected with virulent cultures. When applied to human medicine, the serum failed to do good.

The treatment of pneumonia by the injection of blood-serum from convalescent patients, tried by Hughes and Carter§ has been abandoned as useless and dangerous.

More recent antipneumococcic serums have been experi-

*"Jour. Med. Research," vol. x, p. 228, 1904.

†"Jour. Exp. Med.," Aug. 25, 1905, vii, No. 5.

‡"Berliner klin. Wochenschrift," 1891, Nos. 34 and 35.

§"Therapeutic Gazette," Oct. 15, 1892.

mentally investigated by De Renzi,* Washbourn,† and Pane.‡

Washbourn prepared an *antipneumococcus serum* that protected rabbits against ten times the fatal dose of live pneumococci, in doses of 0.3 c.c. In general, the lines upon which he operated were those of Behring, Marmorek's work with the streptococcus furnishing most of the details. Two cases of human pneumonia seem to have derived some benefit from large doses of this serum. The serums of Pane and De Renzi were not so powerful as those of Washbourn, requiring about 1 c.c. to protect a rabbit.

McFarland and Lincoln § succeeded in immunizing a horse against large doses of a virulent culture of the pneumococcus and obtained a serum, of which 0.5 to 0.25 c.c. protected rabbits from many times the fatal dose.

The antipneumococcic serums thus far produced have given disappointing results in clinical application, probably because their preparation was based upon an antagonism to the toxic products of the pneumococcus rather than upon the destruction of the organism itself. From the most recent investigations upon immunity it is evident that in all diseases in which blood invasion is as frequent as in pneumonia, and the toxicity of the micro-organism so feeble, bacteriolysis is to be aimed at. The most recent experiments upon the serum therapy of pneumonia by Pässler‖ show some gain over the earlier work and give us a more hopeful outlook.

Sanitation.—Pneumonia is undoubtedly a contagious disease. Exactly how infection takes place is not known, but seeing that the infectious agent is in the respiratory tract from which it is easily discharged into the atmosphere during cough, etc., and the facility with which it can then be inhaled by those nearby, it seems justifiable to conclude that the primary entrance of the organism into the body is through the respiratory tract. Wood** has shown that "the organ-

*"Il Policlinico," Oct. 31, 1896, Supplement.

†"Brit. Med. Jour.," Feb. 27, 1897, p. 510.

‡"Centralbl. f. Bakt. u. Parasitenk.," May 29, 1897, XXI, 17 and 18, p. 664.

§"Jour. Am. Med. Assoc.," Dec. 16, 1899, p. 1534.

‖"Deutsches Archiv. für klin. Med.," Bd. LXXXII, Nos. 3, 4, 1905; "Jour. Am. Med. Assoc.," May 13, 1905, p. 1538.

**"Jour. Exp. Med.," Aug. 25, 1905, VII, No. 5, p. 624.

isms in the sputum do not remain long in suspension and die off rapidly under the action of light and desiccation. In sunlight or diffuse daylight the bacteria in such powder die within an hour, and in about four hours if kept in the dark. The danger of infection from powdered sputum may, therefore, be avoided by ample illumination and ventilation of the sick-room in order to destroy or dilute the bacteria, and by the avoidance of dry sweeping or dusting. Articles which may be contaminated and which cannot be cleaned by cloths dampened in a suitable disinfectant should be removed from the patient's vicinity."

"When a person suffering from pneumococcus infection coughs, sneezes, expectorates or talks, particles of sputum or saliva which may contain virulent pneumococci are expelled from the mouth. Such particles remain suspended in the air for a number of hours if the ventilation of the room is good. They may be inhaled by persons in the vicinity of the patient, or they may be deposited upon various articles in the room. Whether suspended in the air or dried upon surrounding objects, the writer's studies show that they become harmless in a very short time, about an hour and a half being the extreme limit, while many of the pneumococci in the spray perish in a few moments, especially if exposed to strong light."

"In the light of these experiments the risk of infection from the pneumococcus is largely confined to those in direct contact with the person whose excreta contain the organism."

PNEUMOCOCCUS (FRIEDLÄNDER*)—BACTERIUM PNEUMONIÆ (ZOPF †)—BACILLUS CAPSULATUS MUCOSUS (FASCHING ‡).

General Characteristics.—An encapsulated, non-motile, non-flagellated, non-sporogenous, non-liquefying, aerobic and optionally anaerobic, non-chromogenic, aerogenic and pathogenic bacillus, staining by ordinary methods, but not by Gram's method.

This organism was discovered by Friedländer in 1883 in the pulmonary exudate from a case of croupous pneumonia, and, being thought by its discoverer to be the cause of that disease, was called the pneumococcus, and later the *pneumobacillus*. The grounds upon which the specificity of the organism was supposed to depend were soon found to be insufficient, and the organism of Friedländer is at present looked upon as one whose presence in the lung is, in most cases, unimportant, though it is sometimes associated with and is probably the cause of a special form of lobular pneumonia, which, according to Stühlern,§ is clinically atypical and usually fatal. Fränkel points out that Friedländer's error in supposing his organism to be the chief parasite in pneumonia depended upon the fact that his studies were made by the plate method, which permitted the discovery of this bacillus to be made more easily than that of the slowly growing and more delicate pneumococcus.

Distribution.—The organism is sometimes found in normal saliva; it is a common parasite of the respiratory apparatus, not infrequently occurs in purulent accumulations, is occasionally found in feces, and sometimes occurs under external saprophytic conditions. Thus it is probably identical with that described as the "capsulated canal-water bacillus" by Mori.‖

Morphology.—Though usually distinctly bacillary in form, the organism is of variable length and when paired sometimes bears a close resemblance to the pneumococcus of Fränkel and Weichselbaum. It frequently occurs in chains of four or more elements and occasionally appears elongate. It is these variations in form that have led to the description of the organism by different writers as a coccus,

*"Fortschritte der Medizin," 1883, 22, 715.

†"Spaltpilze," 1885, p. 66.

‡"Centralbl. f. Bakt.," etc., XII, 1892, p. 304.

§"Centralbl. f. Bakt.," etc. (Originale), Bd. XXXVI, No. 4, p. 493, July 21, 1904.

‖"Zeitschrift für Hygiene," IV, 1888, p. 53.

a bacterium, and a bacillus. It is commonly surrounded by a distinct transparent capsule, hence its name "capsule bacillus" and Bacillus capsulatus mucosus. The organism is non-motile, has no spores and no flagella. It stains well with the ordinary anilin dyes, but does not retain the color when stained by Gram's method.

Cultivation. — **Colonies.**—If pneumonic exudate be mixed with gelatin and poured upon plates, small white spheric colonies appear at the end of twenty-four hours, and spread out upon the surface of the gelatin to form whitish masses of a considerable size. Under the microscope these colonies appear irregular in outline and somewhat granular.

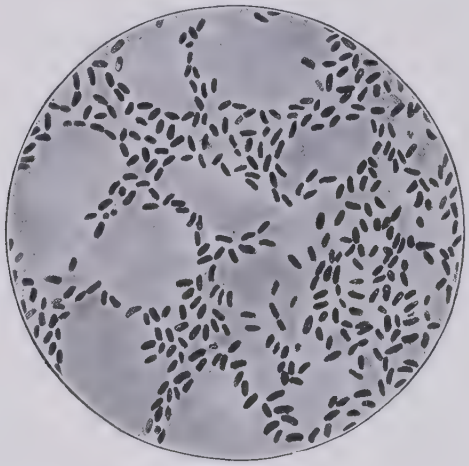


Fig. 87.—Bacterium pneumonicum (modified after Migula).

Bouillon.—There is nothing characteristic about the bouillon cultures of Friedländer's bacillus. The medium is diffusely clouded.

Gelatin Puncture.—When a colony is transferred to a gelatin puncture culture, a luxuriant growth occurs. Upon the surface a somewhat elevated, rounded white mass is formed, and in the track of the wire innumerable little colonies spring up and become confluent, so that a "nail-growth" results. No liquefaction of the gelatin occurs. Gas bubbles not infrequently appear in the wire track. The cultures sometimes become brown in color when old.

Agar-agar.—Upon the surface of agar-agar at ordinary temperatures a luxuriant white or brownish-yellow, smeary, viscid, circumscribed growth occurs.

Blood-serum.—The blood-serum growth is similar to that upon agar.

Potato.—Upon potato the growth is luxuriant, quickly covering the entire surface with a thick yellowish-white layer, which sometimes contains bubbles of gas.

Milk is not coagulated as a rule. Litmus milk is reddened.

Vital Resistance.—The bacillus grows at a temperature as low as 16° C., and, according to Sternberg, has a thermal death-point of 56° C.

Metabolic Products.—Friedländer's bacillus ferments nearly all the sugars with the evolution of much gas. It generates alcohol, acetic and other acids, and both CO_2 and H. It also produces *indol.*

Pathogenesis.—Friedländer found considerable difficulty in producing pathogenic changes by the injection of his bacillus into the lower animals. Rabbits and guinea-pigs were immune to its action, and the only important pathogenic effects that Friedländer observed occurred in mice, into whose lungs and pleura he injected the cultures, with resulting inflammatory lesions.

Curry * found Friedländer's bacillus in association with the pneumococcus in acute lobar pneumonia; in association with the diphtheria bacillus in otitis media associated with croupous pneumonia; and in the

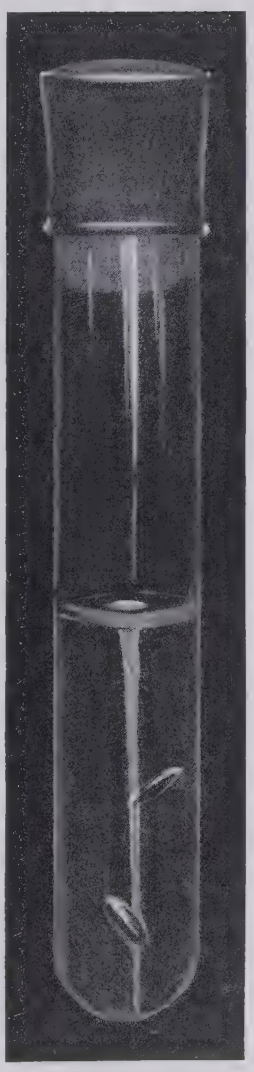


Fig. 88. — Friedländer's pneumobacillus; gelatin stab culture, showing the typical nail-head appearance and the formation of gas bubbles, not always present (Curtis).

* "Jour. Boston Soc. of Med. Sci.," March, 1898, vol. II, No. 8, p. 137.

throat in diphtheria. In pure culture it was obtained from vegetations upon the valves of the heart in a case of acute endocarditis with gangrene of the lung; from the middle ear, in a case of fracture of the skull with otitis media; and from the throat in a case of tonsillitis.

Occasionally Friedländer's bacillus bears an important relationship to lobular or catarrhal pneumonia, an interesting case having been studied by Smith.* The histologic changes in the lung were remarkable in that the "alveolar spaces of the consolidated areas were dilated and for the most part filled with the capsule bacilli." In some alveoli there seemed to be pure cultures of the bacilli; others contained red and white blood-corpuscles; in some there was a little fibrin. The bacillus obtained from this case, when injected into the peritoneal cavity of guinea-pigs, produced death in eleven hours. The peritoneal cavity after death contained a large amount of thick, slimy fluid; the intestines were injected and showed a thin fibrinous exudate upon the surface; the spleen was enlarged and softened, and the adrenals much reddened. Cover-glass preparations from the heart, blood, spleen, and peritoneal cavity showed large numbers of the capsule bacilli.

Howard † has also called attention to the importance of this bacillus in connection with numerous acute and chronic infectious processes, among which may be mentioned croupous pneumonia, suppuration of the antrum of Highmore and frontal sinuses, endometritis, perirenal abscesses, and peritonitis.

Virulence.—The virulence of the organism seems to vary under different conditions. It is sometimes—perhaps usually—harmless for the experiment animals, sometimes produces local inflammatory lesions, sometimes invasion of the circulation and death from sepsis.

CATARRHAL OR BRONCHO-PNEUMONIA.

This form of pulmonary inflammation occurs in local areas, commonly situated about the distribution of a bronchiole. It cannot be said to have a specific micro-organism, as almost any irritating foreign matter accidentally inhaled may cause it. The majority of the cases, however, are

* "Jour. Boston Soc. of Med. Sci.," May, 1898, vol. II, No. 10, p. 174.

† "Phila. Med. Jour.," Feb. 19, 1898, vol. I, No. 8, p. 336.

infectious in nature and result from the aspiration, from higher parts of the respiratory apparatus, of the staphylococci and streptococci of suppuration, Friedländer's bacillus, the bacillus of influenza, and other well-known organisms.

TUBERCULAR PNEUMONIA.

The progress of pulmonary tuberculosis is at times so rapid that the tubercle bacilli are distributed with the softened infectious matter throughout the entire lung or to large parts of it, and a distinct pneumonic inflammation occurs. Such a pneumonia may be caused by the tubercle bacillus, but more frequently depends upon accompanying staphylococci, streptococci, tetragenococci, pneumococci, pneumobacilli, and other organisms accidentally present in a lung in which ulceration and cavity-formation are advanced.

PNEUMONIC PLAGUE.

The pneumonic form of plague is characterized by consolidation of the lung histologically and morbid changes anatomically indistinguishable from pneumococcal and other extensive pulmonary infections.

MIXED PNEUMONIAS.

It frequently happens that pneumonia occurs in the course of influenza or shortly after convalescence from it. In these cases a mixed infection by the influenza bacilli and pneumococci is commonly found. Sometimes pneumococci and staphylococci simultaneously affect the lung, purulent pneumonia with abscess-formation being the conspicuous feature. Almost any combination of the described bacteria may occur in the lungs, producing varying inflammatory conditions, so that it must be left for the student to work out what the particular characteristics of each may be.

Among the mixed forms of pneumonia may be mentioned those called by Klemperer and Levy "complicating pneumonias," occurring in the course of typhoid fever, etc.

II. THE SUB-ACUTE OR CHRONIC LOCAL INFECTIVE DISEASES.

CHAPTER I.

TUBERCULOSIS.

Bacillus Tuberculosis (Koch).*

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-liquefying, non-chromogenic, distinctly aerobic, acid-resisting, purely parasitic, highly pathogenic organism belonging to the higher bacteria. It commonly occurs in the form of slender, slightly curved rods with rounded ends, but not infrequently shows distinct branches.

Tuberculosis is one of the most dreadful and, unfortunately, one of the most common diseases. It is no respecter of persons, but affects alike the young and old, the rich and poor, the male and female, the enlightened and savage, the human being and the lower animals. It is the most common cause of death among human beings, and is common among animals, occurring with great frequency among cattle, less frequently among goats and hogs, and sometimes, though rarely, among sheep, horses, dogs, and cats.

Wild animals under natural conditions seem to escape the disease; but when caged and kept in zoölogic gardens, even the most resistant of them—lions, tigers, etc.—are said at times to succumb to it, while it is the most common cause of death among captive monkeys.

The disease is not limited to mammals, but occurs in a somewhat modified form in birds, and, it is said, even at times affects reptiles, batrachians, and fishes.

The disease has been recognized for centuries; and though, before the advent of the microscope, it was not always clearly differentiated from cancer, it has not only left unmistakable signs of its existence in the early literature of medicine, but has also imprinted itself upon the statute-books of some countries, as the kingdom of Naples, where its ravages were great and the means taken for its prevention radical.

Specific Organism.—Although the acute men of the early

* "Berliner klin. Wochenschrift," 1882, 15.

days of pathology clearly saw that the time must come when the parasitic nature of tuberculosis would be proved, and Klebs, Villemin, and Cohnheim were "within an ace" of its discovery, it remained for Robert Koch to demonstrate and isolate Bacillus tuberculosis, the specific cause of the disease, and to write so accurate a description of the organism and the lesions it produces as to be almost unparalleled in medical literature.

Distribution.—So far as is known, the tubercle bacillus is a purely parasitic organism. It has never been found except in the bodies and discharges of animals affected with tuberculosis, and in dusts of which these are component parts. This purely parasitic nature interferes with the isolation of the organism, which cannot be grown upon the ordinary culture media.

The widespread distribution of tuberculosis at one time suggested that tubercle bacilli were ubiquitous in the atmosphere, that we all inhaled them, and that it was only our *vital resistance* that prevented us all from becoming its victims. Cornet * has, however, shown this to be untrue, as tubercle bacilli exist only in atmospheres contaminated by consumptives. His experiments were made by collecting dusts from streets, sidewalks, houses, rooms, walls, etc., and injecting them into guinea-pigs, whose constant susceptibility to the disease makes them very appropriate for its detection. In this way Cornet showed the bacilli to be present only in dusts with which pulverized sputum was mixed, and found such infectious dusts to be most common where the greatest uncleanliness prevailed.

Our present knowledge of the life-history of the tubercle bacillus, by showing its inability to multiply outside the bodies of animals, the deleterious influence of sunlight upon it, the absence of positive permanent forms, and its sensitivity to temperatures beyond certain extremes, confirms all that Cornet has pointed out, and also explains why, in the course of time, the expectoration of consumptives has not rendered the atmosphere pestilential.

Morphology.—The tubercle bacillus is a slender, rod-shaped organism with slightly rounded ends and a slight curve. It measures from 1.5–3.5 μ in length and from 0.2–0.5 μ in breadth. It commonly occurs in pairs, which may be associated end to end, but generally overlap some-

* "Zeitschrift für Hygiene," v, 1888, pp. 191–331.

what and are not attached to each other. Organisms found in old pus and sputum show a peculiar beaded appearance caused by fragmentation of the protoplasm and the presence of metachromatic granules (Fig. 89). These fragmented

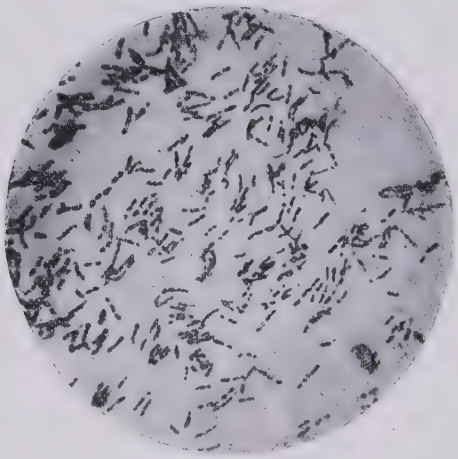


Fig. 89.—Tubercle bacillus in sputum (Fränkel and Pfeiffer).

forms have been thought to be bacilli in the stage of sporulation (see Fig. 90), and Koch originally held this view himself, though later researches have not confirmed it.

Fig. 90.—Tubercle bacilli: 1, Forms suggesting sporulation, because of the presence of large chromophilic granules; 2, forms described as beaded; the open spaces in the fragmented rods are sometimes mistaken for spores; 3, branched forms of the tubercle bacillus sometimes seen in sputum.

The tubercle bacillus forms no endospores. The fragments thought by Koch to be spores are irregular in shape, have ragged surfaces, and are without the high refraction

peculiar to spores. Spores also resist heat strongly, but the fragmented bacilli are no more capable of resisting heat than others.

The bacilli not infrequently present projecting processes or branches, this observation having changed our views regarding the classification of the organism, which is probably erroneously placed among the bacilli, belonging more properly to the higher bacteria and probably being related to the *actinomyces*.

The organism is not motile, and does not possess flagella.

Staining.—The tubercle bacillus is difficult to stain, requiring that the dye used shall contain a mordant (Koch); it is also tenacious of the color once assumed, resisting the decolorizing power of strong mineral acids (Ehrlich).

The peculiarity of staining the bacillus delayed its discovery for a considerable time, but, now that we are

Fig. 91.—Bacillus of tuberculosis showing branched forms with involution (Migula).

familiar with it, gives us a most valuable differential character, few other organisms reacting in the same way.

Koch first stained the bacillus with an aqueous solution of a basic anilin dye, a little potassium hydrate being added as a mordant, subsequently washing the specimen with water and counterstaining it with vesuvin. Ehrlich sub-

sequently modified Koch's method, showing that pure anilin was a better mordant than potassium hydrate, and that the use of a strong mineral acid would remove the color from everything but the tubercle bacillus. This modification of Koch's method given us by Ehrlich is the best method of staining the bacillus.

Staining the Bacillus in Sputum.—As the purpose for which the staining is most frequently performed by the physician is the diagnosis of the disease by demonstrating the bacilli in sputum, that method will be first described.

If one desires to make a very careful examination, it is well to have the patient cleanse the mouth thoroughly upon waking in the morning, and after the first fit of coughing expectorate into a clean, wide-mouthed bottle, the object being to avoid the presence of fragments of food in the sputum.

A better result will be secured if the examination be made on the same day, for if the bacilli are few they occur most plentifully in small flakes of caseous matter, which are easily found at first, but which break up and become part of a granular sediment that forms in decomposed sputum.

The sputum should be poured into a watch-glass and held over a black surface. A number of grayish-yellow, irregular, translucent fragments somewhat smaller than the head of a pin can usually be found. These consist principally of caseous material from the tuberculous tissue, and are the most valuable part of the sputum for examination. One of the fragments is picked up with a pointed match-stick and spread over the surface of a perfectly clean cover-glass or slide. If no such fragment can be found, the purulent part is next best for examination.

The material spread upon the glass should not be too small in amount. Of course, a massive, thick layer will become opaque in staining, but should the layer spread be, as is often advised, "as thin as possible," there may be so few bacilli upon the glass that they are found with difficulty.

The film is allowed to dry thoroughly and is then passed three times through the flame for fixation.

Ehrlich's Method, or the Koch-Ehrlich Method.—Cover-glasses thus prepared are floated, smeared side down, or

immersed, smeared side up, in a small dish of Ehrlich's anilin-water gentian violet solution:

Anilin	4
Saturated alcoholic solution of gentian violet	11
Water	100

and kept in an incubator or paraffin oven for about twenty-four hours at about the temperature of the body. Slides upon which smears have been made can be placed in Coplin jars containing the stain and stood away in the same manner. When removed from the stain, they are washed momentarily in water, and then alternately in 25–33 per cent. nitric acid and 60 per cent. alcohol, until the blue color of the gentian violet is entirely lost. It must be remembered that the action of the strong acid is powerful, and that too long a time must not be allowed for its application. A total immersion of thirty seconds is enough in most cases. After final thorough washing in 60 per cent. alcohol, the specimen is counterstained in a dilute aqueous solution of Bismarck brown or vesuvin, the excess of stain washed off in water, and the specimen dried and mounted in balsam. The tubercle bacilli are colored a fine dark blue, while the pus-corpuscles, epithelial cells, and other bacteria, having been decolorized by the acid, will appear brown.

This method, requiring twenty-four hours for its completion, has fallen into disuse, as it is desirable to know in the briefest possible time whether bacilli are present in the sputum or not.

Ziehl's Method.—Among clinicians, Ziehl's method of staining with carbol-fuchsin has met with just favor. It is as follows: After having been spread, dried, and fixed, the cover-glass is held in the bite of an appropriate forceps (cover-glass forceps), or the slide spread at one end is held by the other end as a handle, and the stain (fuchsin, 1; alcohol, 10; 5 per cent. phenol in water, 100) dropped upon it from a pipet. As soon as the entire smear is covered with stain, it is held over the flame of a spirit lamp or Bunsen burner until the stain begins to volatilize a little. When vapor is observed, the heating is sufficient, and the temperature can be maintained by intermittent heating.

If evaporation take place, a ring of incrusted stain at the edge prevents the prompt action of the acid. To prevent

this, more stain should now and then be added. The staining is complete in from three to five minutes, after which the specimen is washed off with water, and then with a 3 per cent. solution of hydrochloric acid in 70 per cent. alcohol, 25 per cent. aqueous sulphuric, or 33 per cent. aqueous nitric acid solution dropped upon it for thirty seconds, or until the red color is just extinguished. The acid is carefully washed off with water, the specimen dried and mounted in Canada balsam. Nothing will be colored except the tubercle bacilli, which appear red.

Gabbet's Method.—Gabbet modified the method by adding a little methylene-blue to the acid solution, which he makes according to this formula:

Methylene-blue	2
Sulphuric acid	25
Water	75

In Gabbet's method, after staining with carbol-fuchsin the specimen is washed with water, acted upon by the methylene-blue solution for thirty seconds, washed again with water until only a very faint blue remains, dried, and finally mounted in Canada balsam. The tubercle bacilli are colored red; the pus-corpuscles, epithelial cells, and unimportant bacteria, blue.

In cases in which these methods fail to reveal bacilli whose presence is strongly indicated by the clinical signs, a still more exact method of searching for them is to partially digest the sputum with caustic potash, and collect the solid matter with a centrifugal apparatus. When very few bacilli are present in the sputum, this method will often permit them to be demonstrated. When the number is still smaller, it may be possible to show their presence by guinea-pig inoculation when staining methods all fail.

The possible relation that the number of bacilli in the expectoration of consumptives might bear to the progress of the disease has been investigated by Nuttall.* The total quantity of sputum expectorated in twenty-four hours was received in covered, scrupulously clean conical glasses and measured. The proportion of muco-purulent to fluid matter was noted. Depending upon its viscidity and the number of bacilli present in the sputum, a varying amount of 5 per cent. caustic potash solution was added to it (from

* "Bull. of the Johns Hopkins Hospital," May and June, 1891, II, 13.

one-sixth to an equal volume), and after the caustic potash had rendered the sputum perfectly fluid, more or less water was added to dilute the mixture. The sputum, having been measured, was poured into a perfectly clean wide-mouthed bottle containing fine sterilized gravel or broken glass. Rinsings of a measured amount of the caustic potash solution were used to free the conical glass from what matter might remain and were added to the

Fig. 92.—Bacillus tuberculosis in sputum, stained with carbolic fuchsin and aqueous methylene-blue. × 1000 (Ohlmacher).

sputum. The contents of the bottle were agitated in a shaking machine for five minutes, and allowed to stand until the caustic potash solution had had time to act. So soon as the sputum became homogeneous an equal volume of water was added, and the whole was again shaken. The sputum thus treated was of a pale green or yellowish-brown color, and contained only small fragments of elastic tissue. It was allowed to stand from two to four hours, and was then again shaken for five or ten minutes.

By means of a buret, of original design, drops of exactly equal size were delivered and caught upon clean sterile cover-glasses. The drops were subsequently spread into an even film by a very fine platinum wire, while the cover-glass was rotated upon a "turn-table." After spreading, the cover-glasses were laid upon a slightly warmed level brass plate to facilitate drying. After drying, they were coated with a serum film by spraying, and the temperature raised to 80°–90° C. to coagulate the serum and retain the bacteria in place, after which they were carefully stained with carbol-fuchsin and decolorized with a solution of 150 parts of water, 50 parts of alcohol, and 20–30 drops of pure sulphuric acid. Prior to this the cover-glass was washed in three alcohols and subsequently in water, and, if necessary, in acid and alcohol again.

A special arrangement of the microscope was devised for the purpose of counting the bacteria, and the number of bacilli in each drop was estimated with extreme care. The number varied from 472 to 240,000. To estimate the number of bacilli in a given quantity the number of drops to a cubic centimeter is multiplied by the number of bacilli in the drop, and then by the number of cubic centimeters to be estimated.

The method is an ingenious one, but a glance down the columns of figures in the original article is sufficient to show that the number of bacilli is devoid of any practical interest, as is only to be expected when one considers the pathology of the disease and remembers that accidents, such as unusually violent cough one day, modified by the use of sedatives the next, may cause wide variations in the quality, if not in the quantity, of the sputum.

Staining the Bacillus in Urine.—The detection of tubercle bacilli in the urine is sometimes easy, sometimes difficult. The centrifuge should be used and the collected sediment spread upon the glass. If there be no pus or albumin in the urine, it is necessary to add a little white of egg to secure good fixation of the urinary sediment to the glass. The method of staining is the same as that for sputum. The *smegma bacillus* (*q. v.*) is apt to be present in the urine, and the precaution must be taken to wash the specimen with absolute alcohol, so that this bacillus may be decolorized.

Staining the Bacillus in Feces.—It is very difficult to find tubercle bacilli in the feces because of the relatively small number of bacilli and large bulk of feces.

Diagnosis of Tuberculosis.—In all cases where the detection of tubercle bacilli in pus or secretions is a matter of clinical importance, it must be remembered that the quantity of material examined by the staining method is extremely small, so that a few bacilli in a relatively large quantity of matter can easily escape discovery. It is, therefore, important to supplement the microscopic examination by the inoculation of a guinea-pig with a considerable quantity (1–5 c.c.) of the suspected material, and at the end of about three weeks kill it and look for evidences of the disease in its tissues.

Staining the Bacillus in Sections of Tissue.—*Ehrlich's Method for Sections.*—Ehrlich's method must be recommended as the most certain and best. The sections of tissue, embedded in celloidin or paraffin, should be cemented to the slide and then freed from the embedding substance. They are then placed in the stain for from twelve to twenty-four hours and kept at a temperature of 37° C. Upon removal they are allowed to lie in water for about ten minutes to wash away the excess of stain and to soften the tissue (which, if not cemented to the glass, often shrinks and becomes brittle). The washing in nitric acid (20 per cent.) which follows may have to be continued for as long as two minutes. Thorough washing in 60 per cent. alcohol follows, after which the sections can be counterstained, washed, dehydrated in 95 per cent. and absolute alcohol cleared in xylol, and mounted in Canada balsam.

Unna's Method for Sections.—Unna's method is as follows: The sections are placed in a dish of twenty-four-hour-old, newly filtered Ehrlich's solution, and allowed to remain twelve to twenty-four hours at the room temperature or one to two hours in the incubator. From the stain they are placed in water, where they remain for about ten minutes to wash. They are then immersed in acid (20 per cent. nitric acid) for about two minutes, and become greenish-black. From the acid they are placed in absolute alcohol and gently moved to and fro until the pale blue color returns. They are then washed in three or four changes of clean water until they become

almost colorless, and then removed to the slide by means of a section-lifter. The water is absorbed with filter paper, and then the slide is heated over a Bunsen burner until the section becomes shining, when it receives a drop of xylol balsam and a cover-glass.

It is said that sections stained in this manner do not fade so quickly as those stained by Ehrlich's method.

Gram's Method.—The tubercle bacillus stains well by Gram's method and by Weigert's modification of it, but as these are general methods by which many different bacteria are colored, they are ill adapted for differentiation, especially when the other methods are not more difficult.

Isolation and Cultivation.—The best method of obtaining a culture of the tubercle bacillus from sputum, pus, etc., is to inoculate a guinea-pig, allow an artificial tuberculosis to develop, kill the animal after a couple of months, and make cultures from the center of one of the tuberculous glands.

The sputum or other tuberculous material used for inoculation may be injected beneath the skin by a hypodermic syringe, or placed in a little subcutaneous pocket made by snipping the skin of the abdomen with scissors and dissecting it loose so that the fragment is easily introduced. The animal is allowed to live for a month or six weeks, and then killed. The autopsy is performed according to directions already given. An enlarged lymphatic gland with softened contents or a nodule in the spleen is usually selected for the culture. An incision is made into it with a sterile knife, or with a rigid sterile platinum wire, and some of the contents removed and planted upon blood-serum, as recommended by Koch; glycerin agar-agar, as recommended by Roux and Nocard; glycerinized potato, as recommended by Nocard; upon coagulated dogs' blood-serum, as recommended by Smith, or upon coagulated white of egg, as recommended by Dorset. The inoculated tubes must be kept in an incubator at the temperature of 37°–38° C.

Piatkowski* suggests that for the cultivation of the tubercle bacillus and other "acid-proof" organisms, advantage be taken of their ability to resist the action of formaldehyde. The material containing the acid-proof organism is mixed thoroughly with 10 c.c. of water or bouillon

*"Deutsche med. Wochenschrift," June 9, 1904, No. 23, p. 878.

which receives an addition of two or three drops of 40 per cent. formaldehyde or "formalin." After standing from fifteen to thirty minutes, transfers are made to appropriate culture media, when the acid-proof organisms are apt to develop, the others having been destroyed by the formaldehyde.

Blood-serum.—Koch first achieved artificial cultivation of the tubercle bacillus upon blood-serum, upon which the bacilli are first apparent to the naked eye in about two weeks, in the form of small, dry, whitish flakes, not unlike fragments of chalk. These slowly increase in size at the edges, and gradually form small scale-like masses, which under the microscope are found to consist of tangled masses of bacilli, many of which are in a condition of involution.

Kitasato* has published a method by which Koch was able to secure the tubercle bacillus in pure culture, from sputum. After carefully cleansing the mouth the patient is allowed to expectorate into a sterile Petri dish. By this method the contaminating bacteria from the mouth and receptacle are excluded, so that the expectorated material contains only bacteria present in the lungs. The material is carefully washed a great many times in renewed sterile distilled water until all bacteria not inclosed in the mucopurulent material are removed; it is then carefully opened with sterile instruments, and the culture medium—glycerin agar-agar or blood-serum—is inoculated from the center. Kitasato has been able by this method to demonstrate that many of the bacilli ordinarily present in tubercular sputum are dead, although they continue to stain well.

Kitasato's method of washing the sputum has been modified and simplified by Czaplewski and Hensel† in their studies of whooping-cough. Instead of washing the flakes of sputum in water contained in dishes, they shook them in sterile peptone water contained in test-tubes. The shaking in the test-tube being so much more thorough than the washing in dishes, fewer changes of the fluid are necessary, three or four washings being sufficient.

Glycerin Agar-agar.—In 1887 Nocard and Roux‡ gave

* "Zeitschrift für Hygiene," Bd. xi.

† "Centralbl. f. Bakt. u. Parasitenk.," xxii, Nos. 22 and 23, p. 643.

‡ "Ann. de l'Inst. Pasteur," 1887, No. 1.

a great impetus to investigations upon tuberculosis by the discovery that the addition of 4–8 per cent. of glycerin to bouillon and agar-agar made them suitable for the development of the bacillus, and that a much more luxuriant development could be obtained upon such media than upon blood-serum. The growth upon "glycerin agar-agar" (Fig. 93) resembles that upon blood-serum. The growth upon bouillon with added glycerin is also luxuriant. A critical study of the relationship of massive development and glycerin was made by Kimla, Poupé, and Vesely,* who found that the most luxuriant growth occurred when the culture media contained from 5 to 7 per cent. of glycerin. As tubercle bacilli require considerable oxygen for their proper development, they grow only upon the surface of the bouillon, where a thick wrinkled surface growth forms. This growth is rather brittle, and after a time subsides.

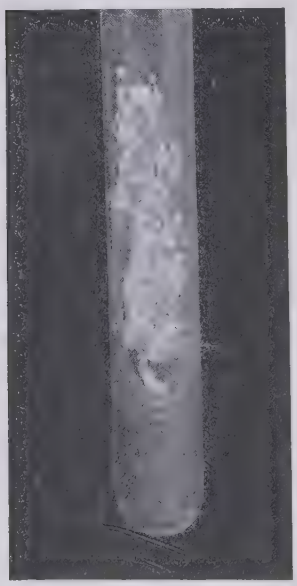


Fig. 93.—Bacillus tuberculosis on "glycerin agar-agar."

Dogs' Blood-serum.—A very successful method of isolating the tubercle bacillus has been published by Smith.† A dog is bled from the femoral artery, the blood being caught in a sterile flask, where it is allowed to coagulate. The serum is removed with a sterile pipet, placed in sterile tubes, and coagulated at 75°–76° C. Smith prefers to use a test-tube with a ground cap, having a small tubular aperture at the end, instead of the ordinary test-tube with the cotton plug. The object is to prevent the contents of the tube from drying during the necessarily long period of incubation.

To the same end the ventilators of the incubator are closed, and a large evaporating dish filled with water is

* "Revue de la Tuberculose," 1898, VI, p. 25.

† "Transactions of the Association of American Physicians," 1898, vol. XIII, p. 417.

stood inside, so that the atmosphere may be constantly saturated with moisture. The tubes are inoculated with bits of tissue the size of a small pea, torn from the tuberculous foci. The fragments of tissue are not crushed or comminuted, but are simply laid upon the undisturbed surface of the blood-serum and then incubated for several weeks. If no growth is apparent after this period, the bit of tissue is stirred about a little and the tube returned to the incubator, where growth almost immediately begins from bacilli scattered over the surface as the bit of tissue was moved about.

Smith secures the tubercle bacillus from sputum by intraperitoneal inoculation of a guinea-pig, and prefers metastatic tuberculous foci to local foci of disease from which to secure material for inoculation. The guinea-pig should not be allowed to die, but should be chloroformed at the end of the third week.

Gelatin.—The tubercle bacillus can be grown in gelatin to which glycerin has been added, but as its development takes place only at 37°–38° C., a temperature at which gelatin is always liquid, its use for the purpose has no advantages.

Potato.—Pawlowski * was able to isolate the bacillus upon potato, but Sander, who found that it could be readily grown upon various vegetable compounds, especially upon acid potato mixed with glycerin, also found that upon such compounds its virulence was lost, and Rosenau † has shown that it can

Fig. 94.—Bacillus tuberculosis; glycerin agar-agar culture, several months old (Curtis)

* "Ann. de l'Inst. Pasteur," 1888, t. VI.
† "Jour. Amer. Med. Assoc.," 1902.

grow upon almost any cooked and glycerinized vegetable tissue. According to French writers, the virulence of the bacillus is not diminished when it grows upon potato. It has also been said that the continued cultivation of the tubercle bacillus upon culture media lessens its parasitic nature, so that in the course of time it can be induced to grow feebly upon the ordinary agar-agar, and that prolonged cultivation destroys its virulence.

Egg Media.—Dorset * recommends the isolation of the tubercle bacillus upon an egg medium, which has the advantage of being cheap and easily prepared, while eggs are

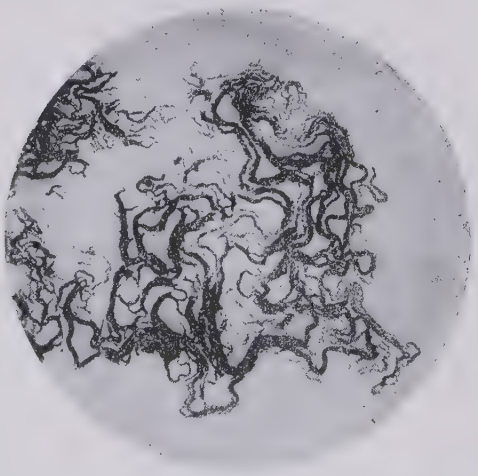


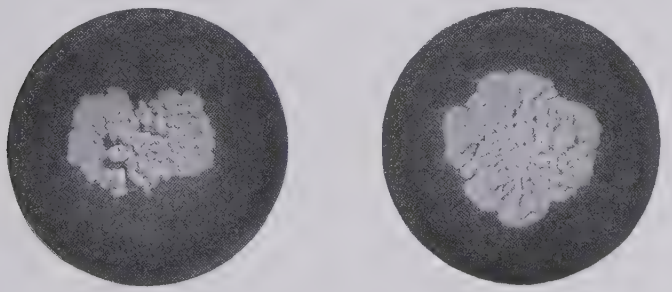
Fig. 95.—Bacillus tuberculosis; adhesive cover-glass preparation from a fourteen-day-old blood-serum culture × 100 (Fränkel and Pfeiffer).

always at hand, and can be made into an appropriate medium in an hour or two. He also claims that the chemic composition of the eggs makes them particularly adapted for the purpose. The medium is prepared by carefully opening the egg and dropping its contents into a wide-mouth sterile receptacle. The yolk is broken with a sterile wire and thoroughly mixed with the white by gentle shaking. The mixture is then poured into sterile tubes, about 10 c.c. in each, inclined in a blood-serum sterilizer, and sterilized and coagulated at 70° C. for two days, the temperature

* "American Medicine," 1902, vol. III, p. 555.

being maintained for four or five hours each day. The medium appears yellowish and is usually dry, so that before using it is well to use a few drops of water to make conditions appropriate for the growth of the tubercle bacillus.

Non-albuminous Media.—It is really surprising to note the extremely simple compounds upon which the tubercle bacillus can be accustomed to grow. Instead of requiring the most concentrated albuminous media, as was once supposed, Proskauer and Beck * have shown that the organism can be made to grow in non-albuminous media containing asparagin, and that it can even be induced to grow upon a mixture of commercial ammonium carbonate, 0.35 per cent.; primary potassium phosphate, 0.15 per cent.; magnesium sulphate, 0.25 per cent.; glycerin, 1.5 per cent. Tuberculin was produced in this mixture.



a *b*

Fig. 96.—Bacillus tuberculosis: *a*, Source, human; *b*, source, bovine. Mature colonies on glycerin-agar. Actual size (Swithinbank and Newman).

Appearance of the Cultures.—Irrespective of the media upon which they are grown, cultures of the tubercle bacillus present certain characteristics which serve to separate them from the majority of other organisms, though insufficient to enable one to certainly recognize them.

The bacterial masses make their appearance very slowly. As a rule very little growth can be observed at the end of a week, and sometimes a month must elapse before the cultures can be described as well grown.

They usually develop more rapidly upon fluid than upon solid media. The growth is invariably and purely aerobic,

* "Zeitschrift für Hygiene," Aug. 10, 1894, XVIII, No. 1.

and the surface growth formed upon liquids closely resembles that upon solids.

The growth is dry and lusterless, coarsely granular, wrinkled, slightly yellowish, and does not extend into the substance of the culture medium. It sometimes extends over the surface of the medium and spreads out upon the contiguous surface of moist glass.

When the medium is moist, the bacterial mass may in rare instances be shining in spots, but it is usually lusterless. When the medium is dry, it is apt to be scaly and almost chalky in appearance.

The organism grows well when once successfully isolated, and, when once accustomed to artificial media, not only lives long (six to nine months) without transplantation, but may be transplanted indefinitely without variation.

In a letter received from Ravenel, I learn that after *five years' continuous cultivation upon artificial media* the tubercle bacillus which he uses for making tuberculin still kills guinea-pigs in three weeks after intraperitoneal inoculation.

Reaction.—The tubercle bacillus will grow upon otherwise appropriate media whether the reaction be feebly acid or feebly alkaline. Human tubercle bacilli scarcely change the reaction of the media in which they grow, but bovine bacilli produce a slight acidity.

Relation to Oxygen.—The tubercle bacillus requires considerable oxygen and therefore grows only upon the surface of the culture media.

Temperature Sensitivity.—The bacillus is sensitive to temperature variations, not growing below 29° C. or above 42° C. Temperatures above 75° C. kill it after a short exposure.

Effect of Light.—It does not develop well in the light, and when its virulence is to be maintained should always be kept in the dark. Sunlight kills it in from a few minutes to several hours, according to the thickness of the mass of bacilli exposed to its influence.

Pathogenesis.—Channels of Infection.—The channels by which the tubercle bacillus enters the body are numerous. A few cases are on record where the micro-organisms have passed through the *placenta*, a tuberculous mother infecting her unborn child. It is not impossible that the passage of bacilli through the placenta in this manner causes the rapid development of tuberculosis after birth, the disease having

remained latent during fetal life, for Birch-Hirschfeld has shown that fragments of a fetus, itself showing no tubercular lesions, but coming from a tuberculous woman, caused fatal tuberculosis in guinea-pigs into which they were inoculated.

The most frequent channel of infection is the *respiratory tract*, into which the finely pulverized pulmonary discharges of consumptives and the dusts of infected rooms and streets enter. Flügge, Laschtschenko, Heyman-Sticher, and Beninde * found that the greatest danger of infection was from the atomized secretions, discharged during cough, from the tuberculous respiratory apparatus. Nearly every one discharges finely pulverized secretions during coughing and sneezing, as can easily be determined by holding a mirror before the face at the time. Even though discharged by consumptives, these atoms of moisture are not infectious, except when tubercle bacilli are present in the sputum. Experiment showed that they usually do not pass further than 0.5 meter from the patient, though occasionally they may be driven 1.5 meters. A knowledge of these facts teaches us that visits to consumptives should not be prolonged; that no one should remain continually in their presence, nor habitually sit within two meters of them; also that patients should always hold a handkerchief before the face while coughing. The rooms occupied by consumptives should also be frequently washed with a disinfecting solution.

Probably all of us at some time in our lives inhale living virulent tubercle bacilli, yet not all suffer from tuberculosis. Personal variations in predisposition seem to account in part for this, as it has been shown that without the formation of tubercles virulent bacilli may sometimes be present for considerable lengths of time in the bronchial lymphatic glands—the dumping-ground of the pulmonary phagocytes.

In order that infection shall occur, it does not seem necessary that the least abrasion or laceration shall exist in the mucous lining of the respiratory tract.

Infection also commonly takes place through the *gastro-intestinal tract* from infected food. At present an overwhelming weight of evidence points to the presence of tubercle bacilli in the milk of cattle affected with tubercu-

* "Zeitschrift für Hygiene," etc., Bd. xxx, pp. 107, 125, 139, 163, 193.

losis. It does not seem necessary that tuberculous ulcers shall be present in the udders; indeed, the bacilli have been demonstrated in considerable numbers in milk from udders without tubercular lesions discoverable to the naked eye.

The meat from tuberculous animals is less dangerous than the milk, because it is nearly always cooked before being eaten, while the milk is generally consumed in the raw state. The ingested bacilli may enter the tonsils and be carried to the cervical lymph-glands, but seem more commonly to reach the intestine, from which they enter the lymphatics, sometimes to produce lesions immediately beneath the mucous membrane, and lead to the later formation of ulcers; but usually to invade the more distant mesenteric lymphatic glands. Nicolas and Descos* and Ravenel† found that when fasting dogs were fed upon soup containing large quantities of tubercle bacilli, they were able to discover the bacilli a few hours afterward in the contents of the thoracic duct. The thoracic duct is sometimes affected, and from such a lesion it is easy to understand the development of general miliary tuberculosis through systemic distribution of bacilli thrown into the circulation. The occasional absorption of tubercle bacilli by the lacteals, and their immediate entrance into the systemic circulation and subsequent deposition in the brain, bones, joints, etc., are supposed to explain primary lesions of these tissues.

Koch believes that human beings are infected only by bacilli from other human beings and his paper upon this subject has stimulated extensive experimentation on the problem. Most authorities believe both human and bovine bacilli to be equally infectious for man. Behring believes that nearly all children become infected by ingesting tubercle bacilli in milk, though a certain predisposition is necessary before the disease can develop. Baumgarten believes that all children harbor bacilli taken in the food, but that the disease does not develop until a certain susceptibility occurs.

Infection also occasionally takes place through the *sexual apparatus*. In sexual intercourse tubercle bacilli from tuberculous testicles can enter the female organs, with resulting bacillary implantation. Sexual infections are usually from the male to the female, primary tuberculosis

* "Jour. de Phys. et Path. gen.," 1902, IV, 910.

† "Jour. Med. Research," X, p. 460, 1904.

of the testicle being more common than of the uterus or ovaries.

Wounds are also occasional avenues of entrance for tubercle bacilli. Anatomic tubercles are not uncommon upon the hands of anatomists and pathologists, most of these growths being tuberculous in nature. Such dermal lesions usually contain few bacilli.

Lesions.—The macroscopic lesions of tuberculosis are too familiar to require a description of any considerable length. They consist of nodules, or collections of nodules, called tubercles, irregularly scattered through the tissues, which are more or less disorganized by their presence and retrogressive changes.

When tubercle bacilli are introduced beneath the skin of a guinea-pig, the animal shows no sign of disease for a week or two, then begins to lose appetite, and gradually diminishes in flesh and weight. Examination usually shows a nodule at the point of inoculation and enlargement of the neighboring lymphatic glands. The atrophy increases, the animal shows a febrile reaction, and dies at the end of a period of time varying from three to twelve weeks. Post-mortem examination usually shows a cluster of tubercles at the point of inoculation, tuberculous enlargement of lymphatic glands both near and remote from the primary lesion, and a widespread tuberculous invasion of the lungs, liver, kidneys, peritoneum, and other organs. Tubercle bacilli are demonstrable in immense numbers in all the invaded tissues. The disease in the guinea-pig is usually more widespread than in other animals because of its greater susceptibility, and the death of the animal occurs more rapidly for the same reason. Intraperitoneal injection of tubercle bacilli in guinea-pigs causes a still more rapid disease accompanied by widespread lesions of the abdominal organs. The animals die in from three to six weeks. In rabbits the disease runs a longer course with similar lesions. In cattle and sheep the infection is commonly first seen in the alimentary apparatus and associated organs, and may be limited to them though primary pulmonary disease also occurs. In man, the disease is chiefly pulmonary, though gastro-intestinal and general miliary tuberculosis is common. The development of the lesions in whatever tissue or animal always depends upon the distribution of the bacilli by the lymph or the blood.

The experiments of Koch, Prudden and Hodenpyl,* and others have shown that when dead tubercle bacilli are injected into the subcutaneous tissues of rabbits, small local abscesses develop in the course of a couple of weeks, showing that the tubercle bacilli possess chemotactic properties. These chemotactic properties seem to depend upon some other irritant than that by which the chief lesions of tuberculosis are caused. When the dead tubercle bacilli, instead of being injected *en masse* into the areolar tissue, are introduced by intravenous injection and disseminate themselves singly or in small groups, the result is quite different, and the lesions closely resemble those caused by the living organisms.

Baumgarten, whose researches were made upon the iris, found that the first irritation caused by the bacillus is followed by multiplication of the fixed connective-tissue cells of the part. These cells increase in number by karyokinesis, and form a minute cellular collection or primitive tubercle. Such leukocytes as occur are of the small mononuclear variety, are of secondary importance, appear later, and are no doubt attracted both by the chemotactic substance shown by Prudden and Hodenpyl to exist in the bodies of the dead bacilli and by the necrotic changes already affecting the tissue in which the tubercle occurs. For reasons not understood, the number of leukocytes varies considerably in different cases. Sometimes there will be enough leukocytes to justify the name "tuberculous abscess"; sometimes they will be completely absent.

The essential toxic substance of the bacillus does not cause the chemotaxis, for when the leukocytes are absent the characteristic coagulation-necrosis persists.

The group of epithelioid cells and leukocytes constituting the primitive tubercle scarcely reaches visible proportions before coagulation-necrosis begins. The cytoplasm of the cells takes on a hyaline character, and appears to become abnormally viscid, contiguous cells tending to fuse. The chromatin of the nuclei becomes dissolved in the nuclear juice and gives a pale but homogeneous appearance to the stained nuclei. Sometimes this nuclear change is only observed very late. There is little karyorrhexis. As the necrosis advances, some of the cells flow together and form large protoplasmic masses—*giant-cells*—which contain as

*"New York Med. Jour.," June 6–20, 1891.

many nuclei as there were component cells. It may be that the nuclei of the giant-cells multiply by karyokinesis after the protoplasmic coalescence, but only one observer, Baumgarten, has found signs of this in giant-cells.

Different writers hold varying opinions concerning the formation and office of the giant-cells. Thus, while I* regard them as degenerative formations, and unimportant

Fig. 97.—Miliary tubercle of the testicle: *a*, Zone of epithelioid cells and leukocytes; *b*, area of coagulation-necrosis; *c*, giant-cell with its processes; peripherally arranged nuclei and necrotic center; *d*, seminiferous tubule (Cameron, in "International Text-book of Surgery").

entities, there are many who believe with Metschnikoff that they are enormous phagocytes. Hektoen† believes that they are active bodies from which cells split off.

Giant-cells are not always formed in tubercles, as the

* "International Medical Magazine," vol. I, No. 10, 1892; vol. III, No. 2, 1894.

† "Journal of Experimental Medicine," vol. III, 1898, p. 21.

necrotic changes are sometimes so rapid and widespread as to convert the whole into a mass of unrecognizable fragments.

Tubercles are constantly avascular,—*i. e.*, in them no new capillary blood-vessels form, as in other inflammatory tissues, and the coagulation-necrosis soon destroys preëxisting capillaries; the avascularity may be a factor in the necrosis of the larger tuberculous masses, though probably playing no important part in the degeneration of the small tubercles, which is purely toxic.

The minute primitive tubercle was first called a *miliary tubercle*, and small aggregations of these "crude tubercles" by Laennec. Tubercles may be developed in any tissue and in any organ. In whatever situation they occur, the component cells are either pushed aside or included in the lesion. In miliary tuberculosis of the kidney it is not unusual to find a tubercle including a glomerule, and resolving its component thrombosed capillaries and epithelium into necrotic fragments. In this way the tissues become disorganized and disintegrated.

As almost all tissues contain a supporting connective-tissue framework, its fibers must be embodied in the new growth. These possess little vitality, but are more resistant than the cells, and, after the cells of a tubercle have been destroyed, may be distinctly visible among the granules, giving the tubercle a reticulated appearance.

As a rule, tubercles progressively increase in size by the invasion of fresh tissue. The tubercle bacillus does not seem to find the necrotic centers of the tubercles adapted to its growth, and most of the bacilli are usually observed at the edges, among the healthy cells, where the nutrition is good. From this position they are occasionally picked up by leukocytes and transported through the lymph-spaces, until the phagocyte falls a prey to its prisoner, dies, and sows the seed of a new tubercle. However, for the spread of tubercle bacilli from place to place phagocytes may not always be necessary, for the bacilli can probably be transported by streams of lymph. It is by the steady advance in necrosis and consolidation that the tissues invaded are destroyed, becoming cheesy and crumbly and forming necrotic masses which, in the lungs, gradually crumble away, the detritus escaping through the air tubes, thus forming cavities. From the beginning of pulmonary

Plate I.

Tuberculosis of the lung: the upper lobe shows advanced cheesy consolidation with cavity-formation, bronchiectasis, and fibroid changes; the lower lobe retains its spongy texture, but is occupied by numerous miliary tubercles.

tuberculosis the process of destruction is greatly accelerated by inspired saprophytic bacteria that live in the necrotic tissue. The patient also suffers from secondary infections, especially by the streptococcus.

Most cases of tuberculosis steadily advance but a certain number may recover.

About the center of a typical tubercle is a zone of reaction in which the reparative tendency of the tissue is usually outweighed by the invasive power of the bacilli.

But if the vital condition of the individual become so changed that the invasive activity of the bacilli is checked or their death brought about, the tubercle begins to cicatrize, and becomes surrounded by a zone of newly formed contracting fibrillar tissue, by which it is circumscribed and isolated. This constitutes recovery from tuberculosis. Sometimes the process of repair is accomplished without the destruction of the bacilli, which are incarcerated and retained. Such a condition is called *latent tuberculosis*, and may at a future time be the starting-point of a new infection.

Virulence.—The virulence of tubercle bacilli varies considerably according to the sources from which they are obtained. Bacilli from different cases are of different degrees of virulence, and bacilli from different animals vary still more. The instructive papers upon "A Comparative Study of Bovine Tubercle Bacilli and of Human Bacilli from Sputum," by Theobald Smith,* and upon "Variation in Virulence of the Bacillus Tuberculosis in Man," by Lartigau,† deserve careful study.

Smith found the bovine bacillus, as a rule, much more virulent for guinea-pigs than the human bacillus. Lartigau found much variation among bacilli secured from the lesions of human tuberculosis. The virulence was tested by employing cultures only for inoculation, and taking of each bacillary mass exactly 5 mg. by weight, suspending it in 5 c.c. of an indifferent fluid until the density was uniform and the microscope showed no clumps, and injecting into rabbits and guinea-pigs, pairs of animals being injected in the same manner, with the same material, at the same time, and being subsequently kept under similar conditions.

* "Journal of Experimental Medicine," 1898, vol. III, p. 459.

† "Journal of Medical Research," vol. VI, No. 1; N. S., vol. I, No. 1, p. 156, July, 1901.

The occurrence of tuberculosis in the inoculated animals was decided by both macroscopic and microscopic tests.

Lartigau found that human tubercle bacilli from different sources produced varying degrees of tuberculosis in animals; that the injection of the same culture in different amounts produces differing results; that the extent and rapidity of development usually corresponds to the virulence of the culture; that doses of 1 mg. of a very virulent culture may induce general tuberculosis in rabbits in a very short time; that 20 mg. of a bacillus of low virulence may fail to produce any lesion in rabbits or guinea-pigs; that no morphologic relationship could be observed between the bacilli and their virulence; that highly virulent bacilli grew scantily on culture media and were short-lived; that bacilli of widely different virulence may be present in any one of the various human tuberculous lesions; that in scrofulous lymphadenitis the bacilli are usually of low virulence; the bacilli in pulmonary tuberculosis with ulceration are of feeble virulence, those of miliary tuberculosis of very great virulence; that the so-called "healed tubercles" of the lung may contain virulent or attenuated bacilli; that individuals suffering from infection with a bacillus of a low grade of virulence may be again infected with extremely virulent tubercle bacilli; that chronic tuberculosis of the bones may contain bacilli of high or low virulence, and that variations in virulence among human tubercle bacilli may possibly sometimes depend, like many other qualities among tubercle bacilli, on peculiarities inherited through serial transmissions in other than human hosts.

Chemistry of the Tubercle Bacillus.—Klebs * found that the tubercle bacillus contains two fatty bodies, one of which, having a reddish color and melting at 42° C., can be extracted with ether. It forms about 20 per cent. by weight of the bacillary substance. The other is insoluble in ether, but soluble in benzole, with which it can be extracted. It melts at about 50° C., and constitutes 1.14 per cent. of the bacillary substance. After removing these fatty bodies the bacilli fail to resist the decolorant action of acids when stained by ordinary methods, so that it seems probable that their acid-resisting power depends upon them.

* "Centralbl. f. Bakt.," 1896, xx, p. 488.

De Schweinitz* showed that it was possible to extract from the tubercle bacillus an acid closely resembling, if not identical with, teraconic acid. It melts at 161°–164° C. and is soluble in ether, water, and alcohol. He thinks the necrotic changes caused by the organism depend upon it.

Ruppel† believes that three different fatty substances are present in the tubercle bacillus, making up from 8 to 26 per cent. by weight. The first can be extracted with cold alcohol, the second with hot alcohol, the third with ether. In addition to the fatty substance Ruppel also found what he believes to be a protamin and calls *tuberculosamin*. It seems to be combined with nucleinic acid, and, indeed, from it he isolated an acid for which he proposes the name *tuberculinic acid*.

Behring‡ found that this acid contained a histon-like body whose removal left chemically pure tuberculinic acid. One gram of this acid is capable of killing a 600-gram guinea-pig when administered beneath the skin. One gram is fatal to 90,000 grams of guinea-pig when introduced into the brain. If injected into tuberculous guinea-pigs it is much more fatal, 1 gram destroying 60,000 when injected subcutaneously and 40,000,000 when injected into the brain.

Levene§ also found free and combined nucleinic acid varying in phosphorus content from 6.58 to 13.19 per cent. He also found a glycogen-like substance that reduced Fehling's solution when heated with a mineral acid.

An important variation in the metabolism of the human and bovine tubercle bacilli has been pointed out by Theobald Smith,‖ who observed that cultures of the two organisms upon glycerine bouillon differed in the induced reaction of the media. The cultures of the bovine bacillus tend toward neutrality, those of the human bacillus toward acidity. This chemical difference is an adjunct to our means of differentiating the two organisms.

Toxic Products.—In 1890 Koch** announced some observations upon the toxic products of the tubercle bacillus

*"Trans. Assoc. of Amer. Phys.," 1897; "Centralbl. f. Bakt.," etc., Sept. 15, 1897, Bd. XXII, p. 200.

†"Zeitschrift für physiol. Chemie," 1899, XXVI.

‡"Berliner klin. Wochenschrift," XXXVI.

§"Jour. of Med. Research," 1, 1901.

‖"Trans. Assoc. Amer. Phys.," 1903, vol. XVIII, p. 109.

**"Deutsche med. Wochenschrift," 1891, No. 343.

and their relation to the diagnosis and treatment of tuberculosis, which at once aroused an enormous though transitory enthusiasm. The observations are, however, of great importance. Koch observed that when guinea-pigs are inoculated with tubercle bacilli, the wound ordinarily heals readily, and soon all signs of local disturbance other than enlargement of the lymphatic glands of the neighborhood disappear. In about two weeks, however, there appears, at the point of inoculation, a slight induration which develops into a hard nodule, ulcerates, and remains until the death of the animal. If, however, in a short time the animals be reinoculated, the course of the local lesion is changed, and, instead of healing, the wound and the tissue surrounding it assumes a dark color, becomes obviously necrotic, and ultimately sloughs away, leaving an ulcer which rapidly and permanently heals without enlargement of the lymph-glands.

This observation was made by injecting cultures of the living bacillus, but Koch observed that the same changes also occur when the secondary inoculation is made with killed cultures of the bacilli.

It was also observed that if the material used for the secondary injections was not too concentrated and the injections not too often repeated (only every six to forty-eight hours), the animals treated improved in condition, and, instead of dying of tuberculosis in from six to ten weeks, continued to live, sometimes (Pfuhl) as long as nineteen weeks.

Tuberculin.—Koch also discovered that a 50 per cent. glycerin extract of cultures of the tubercle bacillus—*tuberculin*—produced the same effect as the dead cultures originally used, and announced the discovery of this substance to the scientific world, in the hope that the prolongation of life observed to follow its use in the guinea-pig might also be true of man.

The active substance of the "tuberculin" seems to be an albuminous derivative (bacterio-proteid) insoluble in absolute alcohol. It is a proteid substance and gives all the characteristic reactions. It differs from the toxalbumins in being able to resist exposure to 120° C. for hours without change. Tuberculin is almost harmless for healthy animals, but extremely poisonous for tuberculous animals, its injection into them being followed either by a violent febrile

reaction or by death, according to the extent of the disease and size of the dose administered.

Preparation of Tuberculin.—The preparation of tuberculin is simple. Flasks (Fig. 98) made broad at the bottom so as to expose a considerable surface of the contained liquid

Fig. 98.—Massive culture of the tubercle bacillus upon the surface of glycerin bouillon, used in the manufacture of tuberculin.

are filled to a depth of about 2 cm. with bouillon containing 4–6 per cent. of glycerin and preferably made with veal instead of beef-infusion. They are inoculated with pure cultures of the tubercle bacillus, care being taken that the bacillary mass floats upon the surface, and are kept in an incubator

at 37° C. In the course of some days a slight surface growth becomes apparent about the edges of the floating bacillary mass, which in the course of time develops into a firm, coarsely granular, wrinkled pellicle. At the end of some weeks development ceases and the pellicle sinks, a new growth sometimes occurring from floating scraps of the original growth.

Some bacteriologists prefer to use small Erlenmeyer flasks for the purpose, but large flasks such as are shown in the illustration, and which may contain from 500 c.c. to 1 liter, are more convenient. The contents of a number of flasks of well-grown cultures are poured into a large porcelain evaporating dish, concentrated over a water-bath to one-tenth their volume, and filtered through a Pasteur-Chamberland filter. This is *crude* tuberculin.

When doses of a fraction of a cubic centimeter of crude tuberculin are injected into tuberculous animals, an inflammatory and febrile reaction occurs. Superficial tuberculous lesions (lupus) sometimes ulcerate and slough away. The febrile reaction is sufficiently characteristic to be of diagnostic value, though tuberculin can only be used with perfect safety as a diagnostic agent upon the lower animals.

From the "crude" or original tuberculin Koch prepared a purified or "*refined*" tuberculin by adding one and one-half volumes of absolute alcohol, stirring thoroughly and standing aside for twenty-four hours. At the end of this time a flocculent deposit will be seen at the bottom of the vessel. The supernatant fluid is carefully decanted and an equal volume of 60 per cent. alcohol poured into the vessel for the purpose of washing the precipitate, which is again permitted to settle, the fluid decanted and the washing thus repeated several times, after which it is finally washed in absolute alcohol and dried in a vacuum exsiccator. The white powder thus prepared is fatal to tuberculous guinea-pigs in doses of 2–10 mg. It is soluble in water and glycerin and gives the proteid reactions. The tuberculin as Koch prepared it is now known as "concentrated" or "Koch's tuberculin," to differentiate it from the "diluted tuberculin" sometimes sold in the shops, which is the same thing so diluted with 1 per cent. aqueous carbolic acid solution that 1 c.c. equals a dose. The dose of the concentrated tuberculin is 0.4–0.5 c.c.; that of the diluted tuberculin, 1 c.c.

Tuberculin Test for Tuberculosis of Cattle.—The febrile

reaction caused by the injection of tuberculin into tuberculous animals is an important adjunct to our means of diagnosticating the disease. For the recognition of tuberculosis in cattle it is easily carried out.

To make a satisfactory diagnostic test the temperature of the animal should be taken every few hours for a day or two before the tuberculin is administered, in order that the normal diurnal and nocturnal variations of temperature shall be known. The tuberculin is then administered by hypodermic injection into the shoulder or flank, and the temperature subsequently taken every two hours for the next twenty-four hours. *A reaction of two degrees beyond that normal to the individual animal is positive of tuberculosis.* After one reaction of this kind the animal will not again react to an equal dose of tuberculin for a number of weeks.

Tuberculin does not exert the slightest influence upon the tubercle bacillus, but acts upon the tuberculous tissue, augmenting the poisonous influence upon the cells surrounding the bacilli, destroying their vitality, and removing the conditions favorable to bacillary growth, which for a time is checked. This action is accompanied by marked hyperemia of the peritubercuIous tissue, with transudation of serum, softening of the tuberculous mass, and its absorption into the blood, a marked febrile reaction resulting from the intoxication.

Virchow, who well understood the action of the tuberculin, soon showed that as a diagnostic and therapeutic agent in man its use was attended by grave dangers. The destroyed tissue was absorbed, but with it some of the bacilli, which, being transported to new tissue areas, could occasion a widespread metastatic invasion of the disease. Old tuberculous lesions which had been encapsulated were sometimes softened and broken down, and became renewed sources of infection to the individual, so that, a short time after an enthusiastic reception tuberculin was placed upon its proper footing as an agent valuable for diagnosis in veterinary practice, but dangerous in human medicine, except in cases of lupus and other external forms of tuberculosis where the destroyed tissue could be readily discharged from the surface of the body.

Many, however, continued to use it, and Petruschky* has

*"Berliner klin. Wochenschrift," 1899, Dec. 18–25.

reported, with careful details, 22 cases of tuberculosis which he claims have been cured by it.

Recently there has been a return to the use of tuberculin for the diagnosis of tuberculosis, it being claimed that by the use of minute doses, several times repeated, the characteristic reaction and a positive diagnosis can be obtained without danger.

Klebs* has made strong claims for his own modifications of tuberculin, known as *antiphthisin* and *tuberculocidin*. According to the experimental studies of Trudeau and Baldwin, however, antiphthisin is only much diluted tuberculin, and exerts no demonstrable influence upon the tubercle bacillus *in vitro*, does not cure tuberculosis in guinea-pigs, and probably inhibits the growth of the tubercle bacillus upon culture media to which it has been added, only by its acid reaction. The preparations are no longer mentioned in the literature except as having failed to cure tuberculosis.

Tuberculin-R.—What appears to be an important modification of tuberculin has been made by Koch,† in the TR or tuberculin-R.

All attempts to produce immunity against the tubercle bacillus by the injection of attenuated cultures, whether dead or alive, fail because of the invariable occurrence of abscesses following their introduction into the cellular tissue, and of nodular growths in the lungs succeeding their injection into the circulation. It seemed as if the fluids of the body could not effect the solution of the bacteria and the liberation of their essential toxic and immunizing constituents.

Koch therefore endeavored to bring about artificial conditions advantageous to the absorption of the bacilli, and for the purpose tried the solvent action of diluted mineral acids and alkalies. The changes thus brought about facilitated absorption, but the absorption of bacilli in this chemically altered condition was not followed by immunity, probably because the chemic composition of tubercle toxin (or whatever one may name the poisonous product of the bacillus) was altered by the reagents.

Tuberculin, with which Koch performed many experiments, was found to produce immunity only against tuberculin, not against bacillary infection.

*"Die Behandlung der Tuberculose mit Tuberculocidin," 1892.

†"Deutsche med. Wochenschrift," 1897, No. 14.

Pursuing the idea of fragmenting the bacilli, or treating them chemically to increase their solubility, Koch found that a 10 per cent. sodium hydrate solution yielded an alkaline extract of the bacillus, which, when injected into animals, produced effects similar to those following the administration of tuberculin, except that they were more brief in duration and more constant in result; but the disadvantage of abscess-formation following the injections remained. The fluid, when filtered, possessed the properties of tuberculin.

Mechanical fragmentation of bacilli had been employed by Klebs in his studies of *antiphthisin* and *tuberculocidin*, and Koch now used it with advantage. He pulverized living, virulent, but perfectly dry bacilli in an agate mortar, in order to liberate the toxic substance from its protecting envelop of fatty acid, triturating only very small quantities of the bacteria at a time.

Having thus reduced the bacilli to fragments, he removed them from the mortar, placed them in distilled water, washed them, and collected the fragments by centrifugation, as a muddy residuum at the bottom of an opalescent, clear fluid. For convenience he named the clear fluid TO; the sediment, TR. TO was found to contain tuberculin. In order to separate the essential poison of the bacteria as perfectly as possible from the irritating tuberculin, the TR fragments were again dried perfectly, triturated once more, re-collected in fresh distilled water, and recentrifugated. After the second centrifugation microscopic examination showed that the bacillary fragments had not yet been resolved into a uniform mass, for when TO was subjected to staining with carbol-fuchsin and methylene-blue it was found to exhibit a blue reaction, while in TR a cloudy violet reaction was obtained.

The addition of 50 per cent. of glycerin had no effect upon TO, but caused a cloudy white deposit to be thrown down from TR. This last reaction showed that TR contained fragments of the bacilli insoluble in glycerin.

In making the TR preparation Koch advises the use of a fresh, highly virulent culture not too old. It must be perfectly dried in a vacuum exsiccator, and the trituration, in order to be thorough, should not be done upon more than 100 mg. of the bacilli at a time. A satisfactory separation of the TR from TO is said only to occur when

the perfectly clear TO takes up at least 50 per cent. of the solid substance, as otherwise the quantity of TO in the final preparation is so great as to produce undesirable reactions.

The fluid is best preserved by the addition of 20 per cent. of glycerin, which does not injure the TR and prevents its decomposition.

The finished fluid contains 10 mg. of solid constituents to the cubic centimeter, and before administration should be diluted with physiologic salt solution (not solutions of carbolic acid). When administering the remedy to man, the injections are made with a hypodermic syringe into the tissues of the back. The beginning dose is $\frac{1}{5000}$ mg., rapidly increased to 20 mg., the injections being made daily.

Experiment showed that TR had decided immunizing powers. Injected into tuberculous animals in too large a dose it produces a reaction, but its immunizing effects were entirely independent of the reaction. Koch's aim in using this preparation in the therapeutic treatment of tuberculosis was to produce immunity against the tubercle bacillus without reactions, by gradual but rapid increase of the dose. In so large a number of cases did Koch produce immunity to tuberculosis by the administration of TR, that he believes it proved beyond a doubt that his observations are correct.

By proper administration of the TR he was able to render guinea-pigs so completely immune that they were able to withstand inoculation with virulent bacilli. The point of inoculation presents no change when the remedy is administered; and the neighboring lymph-glands are generally normal, or when slightly swollen contain no bacilli.

In speaking of his experiments upon guinea-pigs, Koch says:

"I have, in general, got the impression in these experiments that full immunization sets in two or three weeks after the use of large doses. A cure in tuberculous guinea-pigs, animals in which the disease runs, as is well known, a very rapid course, may, therefore, take place only when the treatment is introduced early—as early as one or two weeks after the infection with tuberculosis.

"This rule avails also for tuberculous human beings, whose treatment must not be begun too late. . . . A patient who has but a few months to live cannot expect any value from the use of the remedy, and it will be of

little use to treat patients who suffer chiefly from secondary infection, especially with the streptococcus, and in whom the septic process has put the tuberculosis entirely in the background."

One very serious objection, first urged against commercially prepared TR by Trudeau and Baldwin,* is that it is possible for it to contain unpulverized, and hence still living, virulent tubercle bacilli. Thelling † could not observe any good effect to result from the use of Koch's new tuberculin, and, like Trudeau, found living, virulent bacilli in the preparation secured from Höchst. Many others have since discovered the same danger. In the preparation of the remedy it will be remembered that no antiseptic or germicide was added to the solutions, by which the effects of accidental failure to crush every bacillus could be overcome, Koch having specially deprecated such additions as producing destructive changes in the TR. Until this possibility of danger can be removed, and our confidence that attempts to cure patients may not result in their infection be restored, it becomes a question whether TR can find a place in human medicine, or must remain an interesting laboratory product.

Baumgarten and Walz ‡ find that the administration of tuberculin-R to guinea-pigs is without curative effect. They insist that the results obtained are like those of the old tuberculin; that "small doses are of no advantage, while the larger the doses one employs, the greater are the disadvantages that result from their employment."

Agglutination.—Arloing found that when to homogenized cultures of the tubercle bacillus the serum of a normal goat is added, no change occurs. If, however, the serum is from a goat that has received injections of strong tuberculin or of tubercle bacilli, typical agglutinations, like those of Widal's typhoid test, occur. Thelling § has shown, however, that the effect of the serums of tuberculous individuals is too irregular to be of practical diagnostic importance. It occurs also with the serums of animals injected with Koch's "bacillus-emulsion."

*"Medical News," Aug. 28, 1897.

†"Centralbl. f. Bakt.," etc., July 5, 1902, XXXII, No. 1, p. 28.

‡"Centralbl. f. Bakt. und Parasitenk.," April 12, 1898, XXIII, No. 14, p. 593.

§ *Loc. cit.*

Antitubercle Serums.—Tizzoni and Centanni,* Bernheim,† Paquin,‡ and others have experimented in various ways, hoping that the principles of serum therapy might apply to tuberculosis. Nothing has, however, been achieved. Maragliano's§ antitubercle serum has been used in a very large number of cases in human medicine, and many cures as well as improvements are reported. Behring‖ comments upon it by saying that "Maragliano's tubercle antitoxin contains no antitoxin."

Babes and Proca,** in an experimental research upon the action of an antituberculous serum, claim to have observed a decided specific action.

Maffucci and di Vestea†† found that by injecting guinea-pigs with serum from sheep immunized by injections first of dead, then of living cultures of tubercle bacilli, although no cures were brought about, the vitality of the animals was maintained longer. Unprotected animals died in fifty to fifty-three days; those injected after infection, seventy-four days; and those injected before infection, ninety-one days.

The author ‡‡ made a study of *antituberculin*, prepared by injecting donkeys for a long period with increasing doses of tuberculin. Experiments upon guinea-pigs showed that the serum was powerless to immunize against the tubercle bacillus, or to cure established tuberculosis. The serum, however, had the power of annulling the effects of tuberculin upon tuberculous animals.

De Schweinitz §§ injected cows and horses with increasing quantities of bouillon cultures of a greatly attenuated tubercle bacillus, and thought he found the serum capable of rendering guinea-pigs immune.

Fisch‖‖ immunized a horse against tuberculin-R, hoping

*"Centralbl. f. Bakt.," etc., Bd. XI, p. 82.
†"Centralbl. f. Bakt.," etc., Bd. XV, p. 654.
‡"New York Med. Rec.," 1895.
§"Berliner klin. Wochenschrift," 1895, No. 32.
‖"Fortschritte der Med.," 1897.
**"La Med. Moderne," 1896, p. 37.
††"Centralbl. f. Bakt.," etc., 1896, Bd. XIX, p. 208.
‡‡"Jour. Amer. Med. Assoc.," Aug. 21, 1897.
§§"Centralbl. f. Bakt. und Parasitenk.," Sept. 15, 1897, Bd. XXII, Nos. 8 and 9.
‖‖"Jour. Amer. Med. Assoc.," Oct. 30, 1897.

to produce an antitoxin that might be useful in treating tuberculosis. His experiment resulted in "Antiphthisic Serum, TR," which was claimed to thoroughly immunize guinea-pigs against tuberculosis, to cure tuberculous guinea-pigs in the early stages of the disease, and to neutralize the effects of tuberculin upon tuberculous animals.

Paterson* has suggested a method of immunization against tuberculosis by the use of increasing doses of the serum of a fowl immunized against avian tuberculosis by gradually increased doses of sterilized, attenuated, and virulent cultures of the avian tubercle bacillus. The preparation appears to have met the common fate—oblivion.

From these discordant observations, the more favorable of which are probably the hasty records of inadequate or incomplete experiments, the conclusion that little is to be hoped from immune serums in the treatment of tuberculosis is inevitable.

Prophylaxis.—It is the duty of every physician to use every means in his power to prevent the spread of tuberculous infection in the households under his care. To this end patients should cease to kiss the members of their families and friends; should have individual knives, forks, spoons, cups, napkins, etc., carefully kept apart—secretly if the patient be sensitive upon the subject—from those of the family, and scalded after each meal; should have their napkins and handkerchiefs, as well as whatever clothing or bed-clothing is soiled by them, kept apart from the common wash, and boiled; and should carefully collect the expectoration in a suitable receptacle, that is sterilized or disinfected, without being permitted to dry, as it has been shown that the tubercle bacillus can remain alive in dried sputum as long as nine months. The physician should also give directions for disinfecting the bedroom occupied by a consumptive before it becomes the chamber of a healthy person, though this should be as much the function of the municipality as the disinfection practised after scarlatina, diphtheria, and smallpox.

Boards of health are now becoming more and more interested in tuberculosis, and, though exceedingly slow and conservative in their movements, are disseminating literature with the hope of achieving by volition that which might otherwise be regarded as cruel compulsion.

*"Amer. Medico-Surg. Bull.," Jan. 25, 1898.

So long as tuberculosis exists among men or cattle, it shows that existing hygienic precautions are insufficient. While condemning any unreasonable isolation of patients, I favor the registration of tuberculous cases as a means of collecting accurate data concerning their origin; insist upon the careful domestic sterilization and disinfection of all articles used by the patients; recommend public disinfection of the houses they cease to occupy; and approve of special hospitals for as many, especially of the poorer classes, among whom hygienic measures are almost always opposed, as can be persuaded to occupy them.

BOVINE TUBERCULOSIS.

Bacillus Tuberculosis Bovis.

The tuberculous diseases of the lower animals and especially cattle have lesions closely resembling those of human tuberculosis, and containing bacilli similar both in morphology and in staining reaction to those found in human tuberculosis. The conclusion that they are identical seems inevitable. In his monograph upon tuberculosis Koch called attention to certain morphologic and cultural differences that exist between bacilli obtained from human and from animal tuberculosis, but very little attention was paid to the subject until recently.

It has not yet been determined that any other difference exists between the two bacilli than can be accounted for upon biologic grounds, each organism being modified to accommodate itself to its environment.

Though occasional desultory experiments have been made from time to time, the subject seems to have met its first thorough study at the hands of Theobald Smith,* who carefully compared a series of bacilli obtained from human sputum with another series obtained from cattle, horses, hogs, cats, dogs, and other animals.

His observations form the foundation of the following description of the bovine tubercle bacillus:

Morphology.—The size of the bovine bacillus is quite constant, the individuals being quite short (1–2 μ). They are straight, not very regular in outline, and sometimes of a spindle, sometimes a barrel, and sometimes an oval

* "Trans. Assoc. of Amer. Phys.," 1896, xi, p. 75, and 1898, xiii, p. 417.

shape. The human bacilli, on the other hand, are prone to take an elongate form under artificial cultivation.

Staining.—The bovine bacillus usually stains homogeneously; the human bacillus commonly shows the so-called "beaded appearance," and is more apt to contain rounded, deeply staining bodies suggestive of spores, at or near the ends.

Vegetation.—The human bacillus grows upon dog's serum much more luxuriantly and rapidly than the bovine bacillus.

Pathogenesis.—(a) **Guinea-pigs.**—The bovine bacilli are more virulent than those of human tuberculosis, intraperitoneal inoculation of the former producing death in adult animals in from seven to sixteen days; of the latter, in from ten to thirty-eight days. Subcutaneous inoculation of the bovine bacillus causes death in less than fifty days; of the human bacillus, in from fifty to one hundred days.

(b) **Rabbits.**—Rabbits inoculated into the ear vein with the bovine bacillus die in from seventeen to twenty-one days. Those receiving human bacilli sometimes live several months.

(c) **Cattle.**—Cows and heifers receiving intrapleural and intra-abdominal injections of the human bacilli usually gain in weight and show no symptoms. When examined *post mortem*, circumscribed chronic lesions were found. Those inoculated with the bovine bacillus lose weight, suffer from constitutional symptoms, and show extensive lesions at the necropsy. Two-thirds of the cattle inoculated experimentally with the bovine bacillus die.

Lesions.—In general the lesions produced by the bovine bacillus were rapid, extensive, and necrotic. Many bacilli are present. Those produced by the human bacillus are more apt to be productive, chronic, and unaccompanied by large numbers of bacilli. The bacilli of human tuberculosis produce lesions with many giant-cells; those of bovine tuberculosis, lesions with rapid coagulation-necrosis. The lesions resulting from the intravenous injection of human bacilli into rabbits resembled those observed by Prudden and Hodenpyl* after the intravenous injection of boiled, washed tubercle bacilli.

From these data it is evident that the bovine bacillus is by far the more virulent and dangerous organism. While

*"New York Med. Jour.," June 6–20, 1891.

the human bacillus infects cattle with difficulty, the bovine bacillus infects animals with great readiness.

At the International Congress on Tuberculosis, held in London, 1901, Koch expressed the opinion that bovine tuberculosis was not communicable to man. The matter is of the utmost importance to the medical profession and of far-reaching influence upon many important sanitary measures that bear directly upon the public health.

Koch's opinion, being opposed to all that had been believed before, received almost universal disapproval. The papers by Arloing,* Ravenel,† and Salmon‡ contain evidence showing that under certain conditions bovine tuberculosis can be communicated to man, so that Koch's statement cannot be accepted without hesitation.

Ravenel § has reported three cases of accidental cutaneous inoculation of bovine tuberculosis in man. All were veterinary surgeons who became infected through wounds accidentally inflicted during the performance of necropsies upon tuberculous cattle. The tubercle bacilli were demonstrated in some of the excised cutaneous nodules.

Theobald Smith ‖ in studying three cases of supposed food infection found what corresponded biologically with the human rather than the bovine bacillus.

In a later paper Koch ** analyzes the cases usually selected from the literature to prove the communicability of bovine tuberculosis to man, and seems to show that not one of the cases really proves what is claimed for it, and that the subject requires further careful investigation and demonstration before it will be possible to express any positive opinion in regard to it. The matter must, therefore, at present remain "not proven."

* "Lyon Méd.," Dec. 1, 1901.

† "Univ. of Pa. Bulletin," XIV, p. 238, 1901; "Lancet," Aug. 17 and 19, 1901; "Medicine," July and Aug., 1902, vol. VIII.

‡ Bull. No. 33, Bureau of Animal Industry, U. S. Dept. of Agriculture, 1901.

§ "Phila. Med. Jour.," July 21, 1900.

‖ "Amer. Jour. Med. Sciences," Aug., 1904, vol. CXXVIII, No. 389, p. 216.

** Eleventh International Congress for Tuberculosis, Berlin, 1902.

FOWL TUBERCULOSIS.

Bacillus Tuberculosis Avium.

The occasional spontaneous occurrence of tuberculosis in chickens, parrots, ducks, and other birds, observed as early as 1868 by Roloff * and Paulicki,† was originally attributed to Bacillus tuberculosis hominis, but the work of Rivolta,‡ Mafucci,§ Cadio, Gilbert and Roger, ‖ and others has shown

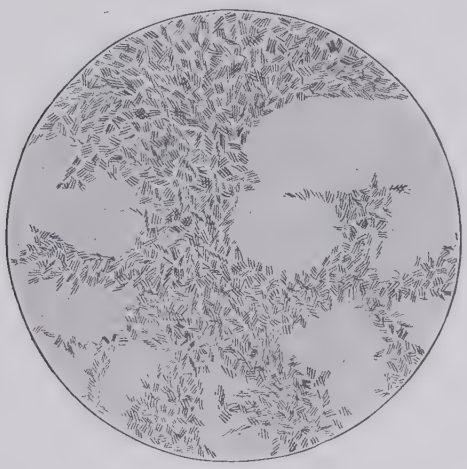


Fig. 99.—Bacillus tuberculosis avium.

that, while very similar to it in many respects, the organism found in the avian diseases has distinct peculiarities which make it a different variety, if not a separate species. Cadio, Gilbert, and Roger succeeded in infecting fowls by feeding them upon food containing tubercle bacilli, and keeping them in cages in which dust containing tubercle bacilli was placed. The infection was aided by lowering the temperature of the birds with antipyrin and lessening their vitality by starvation.

* "Mag. f. d. ges. Tierheilkunde," 1868.
† "Beitr. zur vergl. Anat.," Berlin, 1872.
‡ "Giorn. anat. fisiol. e path.," Pisa, 1883
§ "Zeitschrift für Hygiene," Bd. XI.
‖ "La Semaine medicale," 1890, p. 45.

Morphologic Peculiarities.—Morphologically, the organisms found in avian tuberculosis is similar to that found in the mammalian disease, but is a little longer and more slender, with more marked tendency to club and branched forms. Fragmented and beaded forms occur as in the human tubercle bacilli.

Staining.—The avian bacillus stains in about the same manner as the human and bovine bacilli and has an equal resistance to the decolorant effect of acids.

Cultivation.—Marked rapidity and luxuriance of growth are characteristic of the avian bacillus, which grows upon agar-agar and ordinary bouillon prepared without glycerin. The growth also lacks the dry quality characteristic of cultures of the human and bovine bacilli. Old cultures of the bacillus of fowl tuberculosis turn slightly yellow.

Thermic Sensitivity.—The bacillus also differs in its thermic sensitivity and will grow at 42°–45° C. quite as well as at 37° C., while the growth of the human and mammalian bacilli ceases at 42° C. Moreover, growth at 43° C. does not attenuate its virulence. The thermal death-point is 70° C. Upon culture media it is said to retain its virulence as long as two years.

Pathogenesis.—Birds are the most susceptible animals for experimental inoculation, the embryos and young being more susceptible than the adults; *guinea-pigs are quite immune*, or after inoculation develop cheesy nodes, but do not die. Artificial inoculation can be made in the subcutaneous tissue, in the trachea, and in the veins; never through the intestine. After inoculation the birds die in from one to seven months. The chief seat of the disease is the liver, where cellular (lymphocytic) nodes, lacking the central coagulation and the giant-cell formation of mammalian tuberculosis, and enormously rich in bacilli, are found. The disease never begins in the lungs, and the fowls that are diseased never show bacilli in the sputum or in the dung.

Rabbits are easily infected, an abscess forming at the seat of inoculation, nodules forming later in the lungs, so that the distribution is quite different from that seen in birds. It is probable that the avian bacillus occasionally infects man.

The possibility that this bacillus is derived from the same stock as the tubercle bacillus is strengthened by the experi-

ments of Fermi and Salsano,* who succeeded in increasing its virulence until it became fatal to guinea-pigs by adding glucose and lactic acid to the cultures inoculated.

BACILLI RESEMBLING THE TUBERCLE BACILLUS.

It is not improbable that the bacilli of human, bovine, and avian tuberculosis are closely related to one another and, together with a few other micro-organisms of similar morphology and staining peculiarities, have all a common ancestry and are descended from the same original stock. The most important of these similar organisms are *Bacillus lepræ* (*q. v.*), *Bacillus smegmatis*, and *Moeller's grass bacillus*.

Bacillus Smegmatis.

Alvarez and Tavel,† Matterstock,‡ Klemperer and Bittu,§ Cowie,‖ and others have described peculiar bacilli in smegma taken from the genitals of man and the lower animals, as well as from the moist skin in the folds of the groin, the axillæ, and the anus. They are also sometimes found in urine, and occasionally in the saliva and sputum.

Morphology and Staining.—The organisms are of somewhat variable morphology, but in general resemble the tubercle bacillus, stain with carbol-fuchsin as does the tubercle bacillus, and resist the decolorant action of acids. They are, however, decolorized by absolute alcohol, though Moeller declares the smegma bacillus to be absolutely alcohol-proof, as well as acid-proof, and admits no tinctorial difference between it and the tubercle bacillus. The bacillus, being about the size and shape of the tubercle bacillus, is very readily mistaken for it, and its presence in cases of suspected tuberculosis of the genito-urinary apparatus, and in urine and other secretions in which it is likely to be present, may lead to considerable confusion. The final differentiation may have to rest upon animal inoculation.

Cultivation.—The cultivation of the smegma bacillus is

* "Centralbl. f. Bakt.," etc., xii, 750.

† "Archiv de Physiol. norm. et Path.," 1885, No. 7.

‡ "Mittheil. aus d. med. Klin. d. Univ. d. Würzburg," 1885, Bd. vi.

§ "Virchow's Archives," v, 103.

‖ "Journal of Experimental Medicine," vol. v, 1900–01, p. 205.

difficult and was first achieved by Czaplewski.* Doutrelepont and Matterstock cultivated it upon coagulated hydrocele fluid, but were unable to transplant the growth successfully.

Novy † recommends the cultivation of the smegma bacillus by inoculating a tube of melted agar-agar cooled to 50° C. with the appropriate material, and mixing with it about 2 c.c. of blood withdrawn from a vein of the arm with a sterile hypodermic syringe. The blood-agar mixture is poured into a sterile Petri dish and set aside for a day or two at 37° C. The colonies that form are to be examined for bacilli that resist decolorization with acids.

Moeller ‡ found it comparatively easy to secure cultures of the smegma bacillus by a peculiar method. To secure small quantities of human serum for the purpose of investigating the phenomena of agglutination, he applied small cantharidal blisters to the skins of various healthy and other men, and found large numbers of acid-proof bacilli in the serum saturated with epithelial substance, that remained after most of the serum had been withdrawn. He removed the skin covering from the blister, placed it in the remaining serum, and kept it in the incubator for three or four days, after which he found a dry, floating scum, which consisted of enormous numbers of the bacilli, upon the serum. From this growth he was subsequently able to start cultures of the smegma bacillus upon glycerin agar-agar. Human blood-serum is thus found to be the best medium upon which to start the culture.

Agar.—A culture thus isolated grew upon all the usual culture media. Upon glycerin agar, at 37° C. the colonies appeared as minute, dull, gray-white, dry, rounded scales which later became lobulated and velvety. At room temperature the dry appearance of the growth was retained. The water of condensation remains clear.

Potato.—On potato the growth was luxuriant, grayish, and dull.

Milk.—Milk is said to be an exceptionally good medium, growth taking place in it with rapidity and without coagulation.

* "Münchener med. Wochenschrift," 1897.

† "Laboratory Work in Bacteriology," 1899.

‡ "Centralbl. f. Bakt. u. Parasitenk." (Originale), Bd. xxxi, No. 7, p. 278, March 12, 1902.

Bouillon.—The growth forms a dry white scum upon the surface, the medium remaining clear.

Pathogenesis.—So far as is known, the smegma bacillus is a harmless saprophyte.

Moeller's Grass Bacillus.

Bacilli found in milk, butter, timothy hay, cow-dung, etc., which stain like the tubercle bacillus and may be mistaken for it, have been described by Moeller.* The organisms so closely resemble the tubercle bacillus that guinea-pig inoculations must be resorted to in cases of doubt, but as some of these organisms sometimes kill the guinea-pigs after a month or two, and as small nodules or tubercles may be present in the mesentery, peritoneum, liver, lung, etc., of such animals, the diagnosis may have to be subjected to the further confirmation of a histologic examination of the lesions in order to exclude tuberculosis. In cases of this kind it should not be forgotten that the tubercle bacillus can be present in the substances mentioned, so that the exact differentiation becomes a very fine one. An instructive study of these organisms has been made by Abbott and Gildersleeve,† who, in an elaborate work upon the "Etiological Significance of the Acid-resisting Group of Bacteria, and the Evidence in Favor of their Botanical Relation to Bacillus Tuberculosis," a work that gives complete references to the literature of the subject, come to the following conclusions:

1. That the majority of the acid-resisting bacteria may be distinguished from true tubercle bacilli by their inability to resist decolorization by a 30 per cent. solution of nitric acid in water.

2. That some of the acid-resisting bacteria are capable of causing in rabbits and guinea-pigs nodular lesions suggestive of tubercles; that these lesions, while often very much like tubercles in their histologic structure, may nevertheless usually be distinguished from them by the following peculiarities:

(*a*) When occurring as a result of intravenous inoculation, they are always seen in the kidneys, only occasionally in the lungs, and practically not at all in the other organs.

* "Deutsche med. Zeitung," 1898, p. 135; "Deutsche med. Wochenschrift," 1898, p. 376, etc.

† "Univ. of Pa. Bulletin," June, 1902.

(*b*) They constitute a localized lesion, having no tendency to dissemination, metastasis, or progressive destruction of tissue by caseation.

(*c*) They tend to terminate in suppuration or organization rather than in progressive caseation, as is the case with true tubercles.

(*d*) They are more commonly and conspicuously marked by the actinomyces type of development of the organisms than is the case with true tubercles, and these actinomycetes are less resistant to decolorization by strong acid solutions than are those occasionally seen in tubercles.

3. That by subcutaneous, intravenous, and intrapulmonary inoculation of hogs (4) and calves (15) the typical members of the acid-resisting group are incapable of causing lesions in any way suggestive of those resulting from similar inoculations of the same animals with true tubercle bacilli.

4. That though occasionally present in dairy products, they are to be regarded as of no significance, etiologically speaking, but may be considered as accidental contaminations from the surroundings, and not as evidence of disease in the animals.

5. That the designation "bacillus" as applied to this group of bacteria and to the exciter of tuberculosis is a misnomer; they are more correctly classified as actinomyces.

Isolation and cultivation of these organisms is easy, and more than any other measure serves to differentiate them from the tubercle bacillus, as they grow upon nearly all the culture media with rapidity and luxuriance.

PSEUDO-TUBERCULOSIS.

Bacillus Pseudo-tuberculosis.

Pfeiffer,* Malassez and Vignal,† Eberth,‡ Chantemesse,§ Charrin, and Roger‖ have all reported cases of so-called pseudo-tuberculosis occurring in guinea-pigs, and characterized by the formation of cellular nodules in the liver and kidneys much resembling miliary tubercles. Cultures made from them showed the presence of a small motile bacillus

* "Bacilläre tuberculose, u. s. w.," Leipzig, 1889.

† "Archiv de Physiol. norm. et Path.," 1883 and 1884.

‡ "Virchow's Archiv," Bd. CII.

§ "Ann. de l'Inst. Pasteur," 1887.

‖ "Compte-rendu de l'Acad. des Sci.," Paris, t. CVI.

which could easily be stained by ordinary methods (Fig. 100). When introduced subcutaneously into guinea-pigs, the original disease was reproduced.

Morphology and Cultivation.—Bacillus pseudo-tuberculosis is characterized by Pfeiffer as follows: The organism is rod-shaped, the rods varying in length (0.4 to 1.2 μ) and sometimes united in chains. They may be almost round, and then resemble diplococci. They stain by ordinary methods, but not by Gram's method. They are motile and

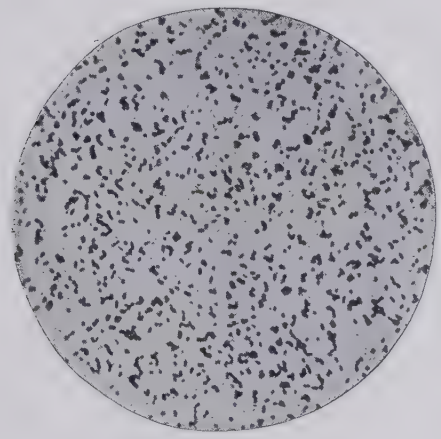


Fig. 100.—Bacillus pseudo-tuberculosis from agar-agar. × 1000 (Itzerott and Niemann).

have flagella like the typhoid and colon bacilli. They form no spores. Upon gelatin and agar-agar circular colonies with a dark nucleus surrounded by a transparent zone are formed. In gelatin punctures the bacilli grow all along the line of puncture and form a surface growth with concentric markings. The gelatin is not liquefied. The bacilli grow readily upon agar and on potato, but without characteristic appearances. In bouillon a diffuse turbidity occurs, with floating and suspended flakes. Milk is not altered.

Pathogenesis.—The bacillus is fatal to mice, guinea-pigs, rabbits, and hares and other rodents in about twenty days after inoculation. At the seat of inoculation an abscess develops, the neighboring lymphatic glands enlarge and caseate, and nodules resembling tubercles form in the internal organs. Similar bacilli studied by Pfeiffer were isolated from a horse supposed to have glanders.

CHAPTER II.

LEPROSY.

Bacillus Lepræ (Hansen).*

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, purely parasitic, acid-resisting bacillus, pathogenic only for man.

Leprosy very early received attention and study. Moses included in the laws to the people of Israel rules for its diagnosis, for the isolation of the sufferers, for the determination of recovery, and for the sacrificial observances to be fulfilled before the convalescent could once more mingle with his people. The Bible is replete with miracles wrought upon lepers, and during the times of biblical tradition it seems to have been an exceedingly common and malignant disease. Many of the diseases called leprosy in the Bible were, however, in all probability less important parasitic skin affections.

Distribution.—At the present time, although we hear very little about it in the northern United States, leprosy is a widespread disease and exists much the same as it did several thousand years ago in Palestine, Syria, Egypt, and the adjacent countries, and is common in China, Japan, and India. South Africa has many cases, and Europe, especially Norway, Sweden, and parts of the Mediterranean coast, a considerable number. In certain islands, especially the Sandwich and Philippine Islands, it is endemic. In the United States the disease is uncommon, the Southern States and Gulf coast being chiefly affected.

A commission of the Marine-Hospital Service, formed for the purpose of investigating the prevalence of leprosy, in 1902 reported 278 existing cases in the United States. Of these, 155 occurred in the State of Louisiana. The other States with numerous cases were California, 24; Florida, 24; Minnesota, 20; and North Dakota, 16. No other State had more than 7 (New York). Of the cases, 145 were American born, 120 foreign born, the remainder uncertain.

* "Virchow's Archives," 1879.

Etiology.—The cause of leprosy is without doubt the lepra bacillus, discovered by Hansen in 1879, and subsequently clearly described by Neisser.

Though the lepra bacillus has certain features—as its resistance to acids—in common with the tubercle bacillus, there is not the slightest evidence of any real identity.

Morphology.—The bacillus is about the same size as the tubercle bacillus—perhaps a little shorter and stouter—and lacks the slight curve of the latter. Its protoplasm commonly presents open spaces or fractures, giving it a

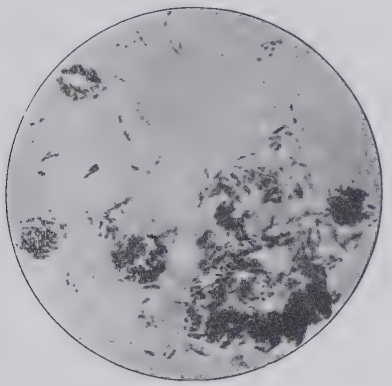


Fig. 101.—Lepra bacilli. Smear from a lepra node stained with carbol-fuchsin (Kolle and Wassermann).

beaded appearance, like the tubercle bacillus. The organism occurs singly or in irregular groups. There is no characteristic grouping and filaments are unknown. The bacillus is not motile and has no flagella and no spores.

Staining.—It stains in very much the same way as the tubercle bacillus, but permits of a more ready penetration of the stain, so that the ordinary aqueous solutions of the anilin dyes color it quite readily. The property of retaining the color in the presence of the mineral acids also characterizes the lepra bacillus, and the methods of Ehrlich, Gabbet, and Unna, for staining the tubercle bacillus, can be used for its detection. It stains well by Gram's method and by Weigert's modification of it, by which beautiful tissue specimens can be prepared.

Czaplewski found that the bacillus which he cultivated and supposed to be the lepra bacillus stained well with Löffler's methylene-blue, and with the aqueous solutions of the anilin dyes. It also stains by Gram's method, and has the same resisting power to the decolorizing action of mineral acids and alcohol as the lepra bacillus seen in tissue. The young bacilli color homogeneously, but

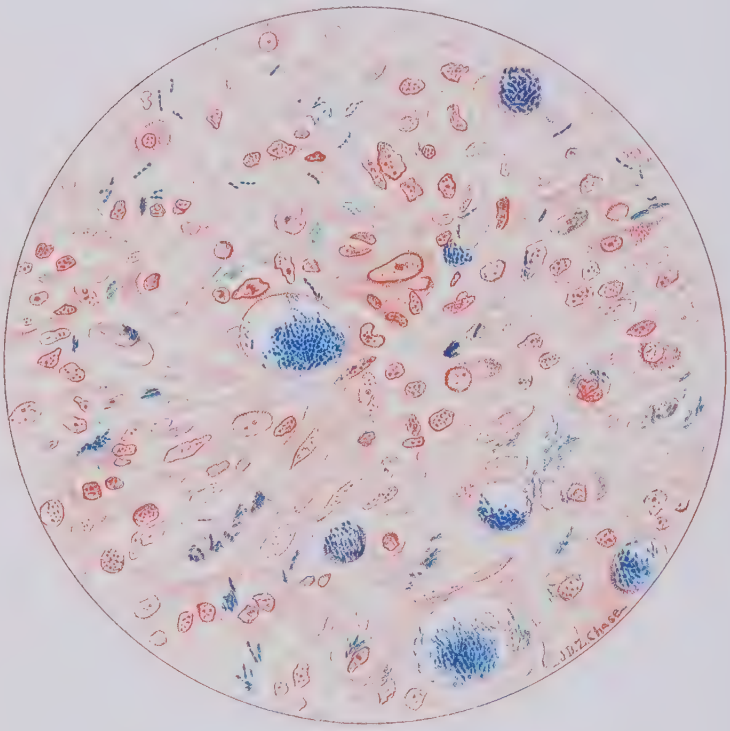


Fig. 102.—Section of one of the nodules from the patient shown in Fig. 103, stained by the Weigert-Gram method to show the lepra bacilli scattered through the tissue and inclosed in the large vacuolated "lepra cells." Magnified 1000 diameters.

older ones are invariably granular. They are usually pointed at the ends when young, but may be rounded or knobbed when older. The more rapidly the bacillus grows, the longer and more slender it appears.

Cultivation.—Bacillus lepræ is a pure parasite and prob-

ably occurs only in places frequented by persons suffering from the disease. That leprous infection occurs less readily than tuberculous infection probably depends upon the fact that lepra bacilli enter the body through cracks or fissures in the skin, while the tubercle bacilli enter through the more accessible respiratory and digestive apparatus.

Many endeavors have been made to cultivate this bacillus upon artificially prepared media, but in spite of modern methods, improved apparatus, and refined media, few claim to have met with success.

Bordoni-Uffredozzi was able to cultivate a bacillus which partook of the staining peculiarities of the lepra bacillus as it appears in the tissues, but differed in morphology. After numerous generations this bacillus was induced to grow upon ordinary culture media. It commonly presented a club-like appearance, which was thought by Baumgarten to depend upon involution. Fränkel found that the bacillus of Bordoni-Uffredozzi possessed none of the essential characteristics of the lepra bacillus except its staining.

Czaplewski * confirmed the work of Bordoni-Uffredozzi, and described a bacillus supposed to be the lepra bacillus, which he succeeded in cultivating from the nasal secretions of a leper.

The bacillus was isolated upon a culture medium consisting of glycerinized serum without the addition of salt, peptone, or sugar. The mixture was poured into Petri dishes, coagulated by heat, and sterilized by the intermittent method.

The secretion, being rich in lepra bacilli, was taken up with a platinum wire and inoculated upon the culture medium by a series of linear strokes. The dishes were then sealed with paraffin and kept in the incubating oven at 37° C.

Numerous colonies, chiefly of Staphylococcus pyogenes aureus and the bacillus of Friedländer, developed and in addition a number of strange colonies, composed of slender bacilli about the size and form of the lepra bacillus.

These colonies were grayish-yellow, humped in the middle, 1–2 mm. in diameter, irregularly rounded, and uneven at the edges. They were firm and could be entirely inverted with the platinum wire, although the consistence was crumbly. They were excavated on the under side.

* "Centralbl. f. Bakt. und Parasitenk.," Jan. 31, 1898, vol. XXIII, Nos. 3 and 4, p. 97.

The colonies that form upon agar-agar are much like those described by Bordoni-Uffredozzi, and appear as isolated, grayish, rounded flakes, thicker in the center than at the edges, and characterized by an irregular serrated border from which a fine irregular network extends upon the medium. These projections consist of bundles of the bacilli.

When a transfer was made from one of these colonies to fresh media, the growth became apparent in a few days and assumed a band-like form, with a plateau-like elevation in the center.

The bacillus thus isolated grew with moderate rapidity upon all the ordinary culture media except potato. Upon blood-serum the growth was more luxuriant and fluid than upon the solid media. Upon coagulated serum the growth was somewhat dry and elevated, and was frequently so loosely attached to the surface of the medium as to be readily lifted up by the platinum wire.

The growth was especially luxuriant upon sheep's blood-serum to which 5 per cent. of glycerin was added. The growth upon the Löffler mixture was also luxuriant.

Upon agar-agar the growth is more meager; it is more luxuriant upon glycerin agar-agar than upon plain agar-agar, the bacterial mass appearing grayish and flatter than upon blood-serum. The growth never extends to the water of condensation to form a floating layer.

The bacillus develops well upon gelatin after it has grown artificially for a number of generations and become accustomed to a saprophytic existence. Upon the surface of gelatin the growth is, in general, similar to that upon agar-agar. In puncture cultures most of the growth occurs upon the surface to form a whitish, grayish, or yellowish wrinkled layer. Below the surface of the gelatin the growth occurs as a thick, granular column. The medium is not liquefied.

In bouillon growth occurs only at the bottom of the tube in the form of a powdery sediment.

Ducrey seems to have cultivated the lepra bacillus in grape-sugar, agar, and in bouillon *in vacuo*. His results need confirmation.

Rost * finds it easy to isolate and cultivate the lepra bacillus upon media free from sodium chloride. The technic of his method is thus described by Rudolph†: "Small lumps of

*"Brit. Med. Jour.," Feb. 22, 1905, and "Indian Med. Gazette," 1905.

†"Medicine," Mar., 1905, p. 175.

pumice stone are washed and then dried in the sun, and then allowed to absorb a mixture of one ounce of meat-extract and two ounces of water. This pumice stone is then placed in wide-mouthed bottles and placed in the autoclave. Each bottle is provided with a stopper through which pass two tubes, the one tube opening into the autoclave and reaching nearly to the bottom of the bottle, and the other leading from the top of the bottle into a condenser adjoining. When the cover of the autoclave is adjusted and the steam admitted, then in the case of each bottle, the steam passes by the one tube to the bottom of the bottle, and rising through the pieces of pumice stone, the steam, carrying with it the volatile constituents of the meat-extract, reaches the condenser by the second tube. The vapor in the condenser yields the salt-free nutrient medium in the proportion of two litres to each ounce of meat-extract originally used. The medium is collected from the condenser in sterilized Pasteur flasks which are kept plunged during the process in a freezing mixture in order to condense some of the volatile alkaloids from the beef that would otherwise escape. The nutrient fluid is now inoculated with the bacillus of leprosy and the flasks kept at 37° for from four to six weeks; at the end of this period when examined the flasks should present a turbid appearance with a stringy white deposit."

Pathogenesis.—Nearly all attempts to infect the lower animals with leprous materials—either purulent matter or scraps of solid tissue from lepers, or with cultures of the several bacilli that have been isolated—have failed.

Melcher and Artmann introduced fragments of lepra nodules into the anterior chambers of the eyes of rabbits, and observed the death of the animals after some months, with what they considered to be typical leprous lesions of all the viscera, especially the cecum; but the recent careful experiments of Tashiro * show that the lower animals are entirely insusceptible to infection with the lepra bacillus, and that when they are inoculated the bacilli persistently diminish in numbers and finally disappear.

Very few instances are recorded in which actual inoculation has produced leprosy in man. Arning was able to experiment upon a condemned criminal, of a family entirely free from the disease, in the Sandwich Islands. Fragments

* 'Centralbl. f. Bakt. u. Parasitenk." (Originale), xxxi, No. 7, p. 276, March 12, 1902.

of tissue freshly excised from a lepra nodule were introduced beneath his skin and the man was kept under observation. In the course of some months typical lesions began to develop at the points of inoculation and spread gradually, ending in general leprosy in about five years.

Sticker* is of the opinion that the primary infection in lepra takes place through the nose, supporting his opinion by observations upon 153 accurately studied cases, in which—

1. The nasal lesion is the only one constant in both the nodular and anesthetic forms of the disease.

2. The nasal lesion is peculiar—*i. e.*, characteristic—and entirely different from all other lepra lesions.

3. The clinical symptoms of lepra begin in the nose.

4. The relapses in the disease always begin with nasal symptoms, such as epistaxis, congestion of the nasal mucous membrane, a sensation of heat, etc.

5. In incipient cases the lepra bacilli are first found in the nose.

Lesions.—The lepra nodes in general resemble tuberculous lesions, but are superficial, affecting the skin and subcutaneous tissues. Rarely they may also occur in the organs. Virchow† has seen a case in which lepra bacilli could be found only in the spleen.

Once established in the body, the bacillus may grow in the connective tissues and produce chronic inflammatory nodes—the analogues of tubercles; or in the nerves, causing anesthesia and trophic disturbances. On this account two forms of the disease—*lepra nodosa* (elephantiasis græcorum) and *lepra anæsthetica*—are described. These forms may occur independently of one another, or may be associated in the same case.

The nodes consist of lymphoid and epithelioid cells and fibers, and are vascular, so that much of the embryonal tissue completes its transformation to fibers without necrotic changes. This makes the disease productive rather than destructive, the lesions resembling new-growths. The bacilli, which occur in enormous numbers, are often found in groups inclosed within the protoplasm of certain large vacuolated cells,—the "lepra cells,"—which seem to be

* "Mittheilungen und Verhandlungen der internationalen wissenschaftlichen Lepra-Konferenz zu Berlin," Oct., 1897, 2. Theil.

† *Ibid.*

partly degenerated endothelial cells. Sometimes they are anuclear; rarely they contain several nuclei (giant-cells). Bacilli also occur in the lymph-spaces and in the nerve-sheaths.

Lepra nodules do not degenerate like tubercles, and the ulceration, which constitutes a large part of the pathology of the disease, seems to be largely due to the injurious action of external agencies upon the feebly vital pathologic tissue.

Fig. 103.—A case of lepra nodosa treated in the Medico-Chirurgical College of Philadelphia, in the service of Prof. John V. Shoemaker.

According to the studies of Johnston and Jamieson,* the bacteriologic diagnosis of nodular leprosy can be made by spreading serum obtained by scraping a leprous nodule, upon a cover-glass, drying, fixing, and staining with carbol-fuchsin and Gabbet's solution as for the tubercle bacillus. In such preparations the bacilli are present in enormous numbers, thus forming a marked contrast to tuberculous skin diseases, in which very few can be found.

* "Montreal Med. Journal," Jan., 1897.

In *anesthetic leprosy* nodules form upon the peripheral nerves, and by connective-tissue formation, as well as by the entrance of the bacilli into the nerve-sheaths, cause irritation, followed by degeneration of the nerves. The anesthesia following the peripheral nervous lesions predisposes to the formation of ulcers, etc., by allowing injuries to occur without detection and to progress without observation. The ulcerations of the hands and feet, with frequent loss of fingers and toes, follow these lesions, probably in the same manner as in syringomyelia.

The disease usually first manifests itself upon the face, extensor surfaces, elbows, and knees, and for a long time confines itself to the skin. Ultimately it sometimes invades the lymphatics and extends to the internal viscera. Death ultimately occurs from exhaustion, if not from the frequent intercurrent affections, especially pneumonia and tuberculosis, to which the conditions predispose.

Specific Therapy.—Carrasquilla's* "leprosy serum" is prepared by injecting the serum separated from blood withdrawn from lepers, into horses, mules, and asses, and, after a number of injections, bleeding the animals and separating the serum. There is no reason for thinking that such a product could have therapeutic value.

Rost† prepares massive cultures of the lepra bacillus, filters them through porcelain, concentrates the filtrate to one-tenth of its volume, and mixes the filtrate with an equal volume of glycerin. The resulting preparation is called *Leprolin* and is supposed to be analogous to tuberculin. With it he has treated a number of lepers at the Leper Hospital at Rangoon, Burmah, many of whom have greatly improved and some of whom seem to be cured. Confirmation of the work by others is greatly desired, and it is too early to judge the merits of the treatment. It is, however, the most promising method yet published.

Sanitation.—While not so contagious as tuberculosis, it has been proved that leprosy is transmissible, and it may be regarded as an essential sanitary precaution that lepers should be segregated and mingle as little as possible with healthy persons. The disease is not hereditary, so that there is no reason why lepers should not marry among themselves. The children should, however, be taken from the parents lest they be subsequently infected.

* "Wiener med. Wochenschrift," No. 41, 1897.

† "Brit. Med. Jour.," Feb. 11, 1905.

CHAPTER III.

CHANCROID.

The Bacillus of Ducrey.

General Characteristics.—A small, ovoid, strepto-bacillus, with rounded, deeply staining ends, non-motile, non-flagellate, non-sporogenous; aerobic and optionally anaerobic, non-chromogenic, staining by ordinary methods but not by Gram's method, cultivable on special media only and pathogenic only for man and certain monkeys.

The chancroid, soft chancre, or non-specific sore, as it is called, is a common venereal affection of both sexes, most frequent among those who give little attention to cleanliness. It is characterized by the appearance of a soft reddish papule, which makes its appearance usually upon the genital organs, rarely upon other parts of the body, soon after the infection and soon becomes transformed to an ugly ulceration whose usual tendency is toward slow and persistent enlargement, though in different cases it may be indolent, active, phagedenic or serpiginous. The inguinal or other near-by lymph nodes early enlarge and soon soften and ulcerate. The disease is, therefore, extremely destructive to the tissues invaded, though no constitutional involvement ever takes place.

Specific Organism.—In 1889 Ducrey* described a peculiar organism whose presence he was able to demonstrate with great constancy, sometimes in pure culture in the lesions of chancroid, and which he believed to be the specific organism of the affection. Up to this time the disease was thought to be non-specific, but since, the bacillus of Ducrey has been looked upon with growing suspicion. Unna† later described an organism resembling that of Ducrey, and the later observations of Krefting,‡ Peterson,§ Nicolle,‖ Cheinisse,** and

*"Congrès. Inter der Dermatol. et de Syphiligr," Paris, 1889, "Compt rendu," p. 229.

†"Monatschr. f. praktische Dermatologie," Bd. XIV, 1892, p. 485.

‡"Archiv. f. Dermatol. u. Syphilol.," 1897, p. 263; 1897, p. 41.

§"Centrabl. f. Bakt.," etc., 1893, XIII, p. 743.

‖"Med. Moderne," Paris, 1893, IV, p. 735.

**"Ann. de Dermat. et de Syphil.," Par., 1894, p. 272.

Davis* have abundantly confirmed the observations of Ducrey and Unna, and proved the identity of the two micro-organisms and their specificity for the disease.

Morphology.—The organism is commonly described as a 'strepto-bacillus.'' It is short and ovoid in shape, and occurs habitually in longer or shorter chains. Each organism measures about $1.5 \times 0.5\ \mu$. The ends are rounded and stain deeply. In pure cultures long undivided filaments, at least twenty times as long as the individual bacilli, are not uncommon. There seems to be no relation between the cells and the bacilli. As a rule, they are free, sometimes they are inclosed in leukocytes.

Staining.—The organisms are somewhat difficult to stain, as they do not retain the color well, giving it up quickly when washed. They do not stain by Gram's method.

Cultivation.—The first successful isolation and cultivation of the organism seems to have been by Bezançon, Griffon and Le Sourd† upon a culture medium consisting of rabbits' blood 1 part, and agar-agar 2 parts. Davis‡ has been equally successful in cultivating the organism upon this medium. His method was as follows:—

''Tubes of two per cent. agar, reaction + 1.5, were melted and mixed with fresh rabbits' blood drawn under aseptic precautions, in the proportion of two-thirds agar to one-third blood, and slanted while in a fluid state. At a later period tubes of rabbits' blood-serum uncoagulated, also rabbits' blood bouillon, one-third blood to two-thirds bouillon, were used, and gave equally satisfactory results. By employing small tubes of freshly-drawn human blood, pure cultures were obtained in several instances from genital lesions direct without any special cleansing of the ulcerated surface. This, I believe, is the best medium for obtaining cultures from a source open to contamination, the fresh blood apparently inhibiting to a certain extent the growth of extraneous organisms.''

No growth takes place upon ordinary culture media under either aerobic or anaerobic conditions.

Colonies.—The colonies appear upon the appropriate media in about twenty-four hours and attain their complete development in about forty-eight hours. They are at first round bright globules, and later become grayish and opaque.

*Jour. Med. Research,'' 1893, IX, p. 401.

†''Ann. de Dermat. et de Syphiligr., 1901, II, p. 1. ‡*Loc. cit.*

They measure one or two millimeters in diameter and never become confluent. They are difficult to pick up with the

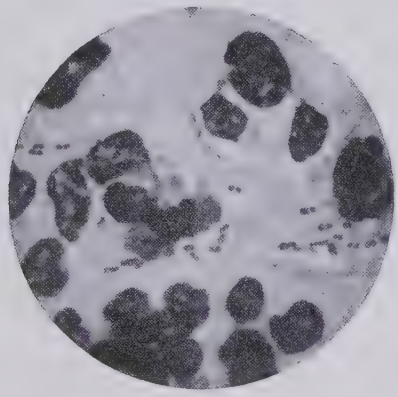


Fig. 104.—Smear of pus of chancroid of penis stained with carbol-fuchsin, and briefly decolorized by alcohol. ×1500 (Davis). (Photomicrograph by Mr. L. S. Brown.)

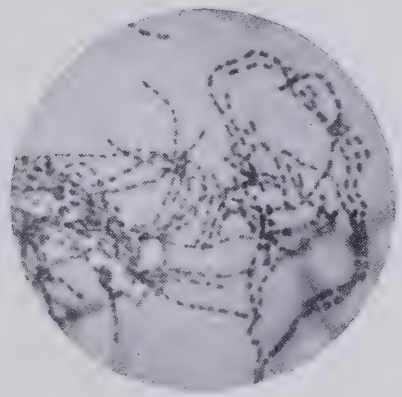


Fig. 105.—Culture from ulceration on monkey resulting from inoculation of culture from a case of chancroid of finger, first generation. Stained with carbol-fuchsin, and briefly decolorized by alcohol. Culture of twenty-four hours' growth in rabbit's bouillon. × 1500 (Davis). (Photomicrograph by Mr. L. S. Brown.)

platinum wire, tending to slide over the smooth surface of the medium.

Vital Resistance.—The organisms seem to possess little vitality, their life in artificial culture being limited to a few

days. Frequent transplantation enabled Davis to carry them on to the eleventh cultural generation.

Pathogenesis.—The organism is pathogenic for man and certain monkeys (macacus), but not for the ordinary laboratory animals. The organism can be found in large numbers in both the genital and extra-genital chancroidal lesions, and usually in small numbers in the pus from chancroidal buboes. It has not been encountered elsewhere. Lenglet* isolated the organism in pure culture, and by inoculation with his cultures reproduced the lesions in man.

*"Bull. Med.," 1898, p. 1051; "Ann. de Dermatol. et de Syph.," 1901, t. II, p. 209.

CHAPTER IV.

GLANDERS.

Bacillus Mallei (Löffler and Schütz).*

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-liquefying, non-chromogenic, aerobic and optionally anaerobic bacillus, pathogenic for man and the lower animals, staining by ordinary methods, but not by Gram's method.

Glanders is an infectious mycotic disease which, fortunately, is almost entirely confined to the lower animals. Only occasionally does it secure a victim among hostlers, drovers, soldiers, and others whose vocations bring them in contact with diseased horses. Several bacteriologists have succumbed to accidental laboratory infection.

Glanders was first known to us as a disease of the horse and ass, characterized by the formation of discrete, cleanly cut ulcers upon the mucous membrane of the nose. The ulcers in the nose of the horse and ass are formed by the breaking down of inflammatory nodules which can be detected in all stages upon the diseased membranes. The ulcers, having once formed, show no tendency to recover, but slowly spread and persistently discharge a virulent pus. The edges of the ulcers are indurated and elevated, their surfaces often smooth. The disease does not progress to any great extent before the submaxillary lymphatic glands begin to enlarge, soften, open, and become discharging ulcers. The lungs may also become infected by inspiration of the infectious material from the nose and throat, and contain small foci of broncho-pneumonia not unlike tubercles in their early appearance. The animals ultimately die of exhaustion.

Specific Organism.—In 1882, shortly after the discovery of the tubercle bacillus, Löffler and Schütz discovered in the discharges and tissues of the disease the specific micro-organism, the glanders bacillus (*Bacillus mallei*, Fig. 106).

* "Deutsche med. Wochenschrift," 1882, 52.

Distribution.—The glanders bacillus does not seem to find conditions outside the animal body suitable for its growth, and probably lives a purely parasitic existence.

Morphology.—The glanders bacillus is somewhat shorter and distinctly thicker than the tubercle bacillus, and has rounded ends. It measures about 0.25–0.4 × 1.5–3 μ, and is slightly bent; coccoid and branched forms sometimes occur. It usually occurs singly, though upon blood-serum, and especially upon potato, conjoined individuals may occasionally be found. Long threads are never formed.

The bacillus is non-motile and has no flagella. The observation of Löffler that the bacilli can be cultivated

Fig. 106.—Bacillus mallei, from a culture upon glycerin agar-agar. × 1000 (Fränkel and Pfeiffer).

after being kept in a dry state for three months makes it appear as if some permanent form (spores) occurs, but none have been observed.

Staining.—The organism can be stained with the watery anilin dye solutions, but not by Gram's method. The bacillus readily gives up the stain in the presence of decolorizing agents, so is difficult to stain in tissues. Löffler accomplished the staining by allowing the sections to lie for some time (five minutes) in the alkaline methylene-blue solution, then transferring them to a solution of sulphuric and oxalic acids—

Concentrated sulphuric acid	2 drops
Five per cent. oxalic acid solution	1 drop
Distilled water	10 c.c.

for five seconds, then to absolute alcohol, xylol, etc. The bacilli appear dark blue upon a paler ground. This method gives very good results, but has been largely superseded by the use of Kühne's carbol-methylene-blue:

Methylene-blue	1.5
Alcohol	10.0
Five per cent. aqueous phenol solution	100.0

Kühne stains the section for about half an hour, washes it in water, decolorizes it carefully in hydrochloric acid (10 drops to 500 c.c. of water), immerses it at once in a solution of lithium carbonate (8 drops of a saturated solution of lithium carbonate in 10 c.c. of water), places it in a bath of distilled water for a few minutes, dips it into absolute alcohol colored with a little methylene-blue, dehydrates it in anilin oil containing a little methylene-blue in solution, washes it in pure anilin oil, not colored, then in a light ethereal oil, clears it in xylol, and finally mounts it in balsam.

Vital Resistance.—The organism grows only between 25° and 42° C. It is killed by exposure to 55° C. for five minutes.

Isolation.—Attempts at the isolation of the glanders bacillus from infectious discharges, by the usual plate method, are apt to fail, on account of the presence of other more rapidly growing organisms.

The best method of isolation seems to be by infecting an animal and recovering the bacillus from its tissues.

The guinea-pig, being a highly susceptible as well as a readily procurable animal, is appropriate for the detection and isolation of the bacillus. When a subcutaneous inoculation of some of the infectious pus is made, a tumefaction can be observed in guinea-pigs in from four to five days. Somewhat later this tumefaction changes to a caseous nodule, which ruptures and leaves a chronic superficial ulcer with irregular margins. The lymph-glands speedily become invaded, and in four or five weeks signs of general infection appear. The lymph-glands, especially of the inguinal region, suppurate, and the testicles frequently undergo the same process. Later the joints are affected with a suppurative arthritis the pus from which contains the bacilli. The

animal eventually dies of exhaustion. No nasal ulcers form in guinea-pigs.

In field-mice the disease is much more rapid, no local lesions being visible. For two or three days the animal seems unwell, its breathing is hurried, it sits with closed eyes in a corner of the cage, and finally, without any other preliminaries, tumbles over on its side, dead.

From the tissues of the inoculated animals pure cultures are easily made. Perhaps the best places from which to secure a culture are the softened nodes which have not ruptured, or the joints.

Diagnosis of Glanders.—Strauss has given us a method which is of great use both for isolating pure cultures of the glanders bacillus, and for making a diagnosis of the disease. But a short time is required. The material suspected to contain the glanders bacillus is injected into the peritoneal cavity of a male guinea-pig. In three or four days the disease becomes established and the testicles enlarge; the skin over them becomes red and shining; the testicles themselves begin to suppurate, and often evacuate through the skin. The animal dies in about two weeks. If, however, it be killed and its testicles examined, the tunica vaginalis testis will be found to contain pus, and sometimes to be partially obliterated by inflammatory exudation. The bacilli are present in this pus, and can be secured from it in pure cultures.

The value of Strauss's method has been somewhat lessened by the discovery by Kutcher,* that a new bacillus, which he has classed among the pseudo-tubercle bacilli, produces a similar testicular swelling when injected into the abdominal cavity; also by Levy and Steinmetz,† who found that Staphylococcus pyogenes aureus was also capable of provoking suppurative orchitis. However, the diagnosis is certain if a culture of the glanders bacillus be secured from the pus in the scrotum.

As the purulent discharges from the noses of horses and other large animals commonly contain very few bacilli, their detection by the use of the guinea-pig inoculation is made much simplified.

For the diagnosis of the disease in living animals, subcutaneous injections of mallein (*q. v.*) are also employed.

Cultivation.—The bacillus is an aerobic and optionally

* "Zeitschrift für Hygiene," Bd. XXI, Heft 1, Dec. 6, 1895.

† "Berliner klin. Wochenschrift," March 18, 1895, No. 11.

anaerobic organism, and can be grown in bouillon, upon agar-agar, better upon glycerin agar-agar, very well upon blood-serum, and quite characteristically upon potato. Gelatin is not liquefied by the glanders bacillus.

Colonies.—Upon 4 per cent. glycerin agar-agar plates the colonies appear upon the second day as whitish or pale yellow, shining round dots. Under the microscope they are brownish-yellow, thick, and granular, with sharp borders.

Bouillon.—In broth cultures the glanders bacillus causes turbidity, the surface of the culture being covered by a slimy scum.

Agar-agar.—Upon agar-agar and glycerin agar-agar the growth occurs as a moist, shining layer.

Fig. 107.—Culture of glanders bacillus upon cooked potato (Löffler).

Blood-serum.—Upon blood-serum the growth is rather characteristic, the colonies along the line of inoculation appearing as circumscribed, clear, transparent drops, which later become confluent and form a transparent layer unaccompanied by liquefaction.

Potato.—The most characteristic growth is upon potato. It first appears in about forty-eight hours as a transparent, honey-like, yellowish layer, developing only at incubation temperatures, and soon becoming reddish-brown in color. As this brown color of the colony develops, the potato for a considerable distance around it becomes greenish-brown.

No other organism is known to produce the same appearance.

Milk.—In litmus milk the glanders bacillus produces acid. A firm coagulum forms and subsequently separates from the clear reddish whey.

Metabolic Products.—Mallein.— Babes,* Bonome,† Pearson,‡ and others have prepared a substance, *mallein*, from cultures of the glanders bacillus, and have employed it for diagnostic purposes. It seems to be useful in veterinary medicine, the reaction following its injection into glandered animals being similar to that caused by the injection of tuberculin into tuberculous animals. The preparation of mallein is simple. Cultures of the glanders bacillus are grown in glycerin bouillon for several weeks, and killed by heat. The culture is then filtered through porcelain, to remove the dead bacteria, and evaporated to one-tenth of its volume. It has also been prepared from potato cultures, which are said to yield a stronger product. The agent is employed exactly like tuberculin, the temperature being taken before and after its hypodermic injection. A febrile reaction of more than 1.5° C. is said to be pathognomonic of the disease.

Pathogenesis.—That the bacillus is the cause of glanders there is no room to doubt, as Löffler and Schütz have succeeded, by the inoculation of horses and asses, in producing the well-known disease.

The goat, cat, hog, field-mouse, wood-mouse, marmot, rabbit, guinea-pig, and hedgehog all appear to be susceptible. Cattle, house-mice, white mice, and rats are immune.

Lesions.—When stained in sections of tissue the bacilli are found in small inflammatory areas. These nodules can be seen with the naked eye scattered through the liver, kidney, and spleen of animals dead of experimental glanders. They consist principally of leukocytes, but also contain numerous epithelioid cells. As is the case with tubercles, the centers of the nodules are prone to necrotic changes, but the cells show marked karyorrhexis, and the tendency is more toward colliquation than caseation. The typical ulcerations depend upon retrogressive changes occurring upon

* "Archiv de Méd. exp. et d'Anat. patholog.," 1892, No. 4.

† "Deutsche med. Wochenschrift," 1894, Nos. 36 and 38, pp. 703, 725, and 744.

‡ "Jour. of Comp. Med. and Vet. Archiv.," Phila., XII, 1891, pp. 411–415.

mucous surfaces, the breaking down of the nodules permitting the softened material to escape. At times the lesions heal with the formation of stellate scars.

Baumgarten * regarded the histologic lesions of glanders as much like those of the tubercle. He first saw epithelioid cells accumulate, followed by the invasion of leukocytes. Tedeschi† was not able to confirm Baumgarten's work, but found the primary change to be necrosis of the affected tissue followed by invasion of leukocytes. The observations of Wright‡ are in accord with those of Tedeschi. He first saw a marked degeneration of the tissue, and then an inflammatory exudation amounting in some cases to actual suppuration.

Virulence.—The organism is said to lose virulence if cultivated for many generations upon artificial media. While this is true, attempts to attenuate fresh cultures by heat, etc., have usually failed.

Immunity.—Leo has pointed out that white rats, which are immune to the disease, may be made susceptible by feeding with phloridzin and causing glycosuria.

Babes has asserted that the injection of mallein into susceptible animals will immunize them against glanders. Some observers claim to have seen good therapeutic results follow the repeated injection of mallein in small doses. Others, as Chenot and Picq,§ find blood-serum from immune animals like the ox to be curative when injected into guinea-pigs infected with glanders.

Pseudoglanders Bacillus.—A bacillus similar in its tinctorial and cultural peculiarities, but not pathogenic for mice, guinea-pigs, or rabbits, was isolated from pus by Selter.‖ The organism was called the pseudoglanders bacillus. A similar one had previously been described by Babes.**

* "Pathologische Mykologie," Braunschweig, 1890.

† "Ziegler's "Beiträge z. path. Anat.," Bd. XIII, 1893.

‡ "Journal of Experimental Medicine," vol. I, No. 4, p. 577.

§ "Compte-rendu de la Soc. de Biol.," March 26, 1892.

‖ "Centralbl. f. Bakt.," etc., Feb. 18, 1902, XXXV, 5, p. 529.

** "Archiv. de med. exp. et d'anat. path.," 1891.

CHAPTER V.

SYPHILIS.

Although syphilis is otherwise well known, its specific cause has not yet been discovered. Whether it be due to a protozoan parasite, as has long been supposed, and the discovery of which has recently been claimed by Max Schüller, or to some bacillus such as has been described by Lustgarten or van Niessen, future investigation must decide. The fact that the disease was not successfully communicated to any of the lower animals until 1903, when Metschnikoff and Roux* succeeded in infecting a chimpanzee, and this work was confirmed by Lassar,† has markedly interfered with its successful etiologic investigation.

Metschnikoff and Roux‡ also have since found it possible to infect macaques with syphilis, though in them the disease was always mild and local. Passing the virus from the macaques to the chimpanzee, they found it attenuated.

Bacillus of Lustgarten.

General Characteristics.—An elongate bacillus known only in preparations stained by a peculiar method and susceptible of neither cultivation nor successful animal inoculation. Very probably identical with the smegma bacillus when in the discharges, and with the tubercle bacillus when in internal lesions.

In 1884 and 1885 Lustgarten§ published a description of peculiar bacilli which he found by staining syphilitic tissues by a peculiar method and assumed to be the cause of the disease. The method of staining is somewhat complicated, and requires that sections of tissue be stained in Ehrlich's anilin water gentian-violet solution for from twelve to

*"Ann. de l'Inst. Pasteur," Dec., 1903, p. 809.

†"Berl. klin. Wochenschrift," 1903, p. 1189.

‡"Annales," Jan., 1904.

§"Wiener med. Wochenschrift," 1884, p. 47, and "Wiener med. Jahrb.," 1885.

twenty-four hours at the temperature of the room, or for two hours at 40° C.; washed for a few minutes in absolute alcohol; immersed for about ten seconds in a 1.5 per cent. permanganate of potassium solution, then placed in an aqueous solution of sulphurous acid for one or two seconds, thoroughly washed in water, dehydrated in alcohol, cleared in oil of cloves, and finally mounted in Canada balsam dissolved in xylol.

If pus or other discharges from syphilitic lesions are to be examined, the cover-glasses spread with the material are stained in the same manner, except that for the first washing distilled water instead of absolute alcohol is used.

This method underwent a modification in the hands of De Giacomi,* who preferred to stain the cover-glasses in a hot anilin-water-fuchsin solution for a few moments, or sections in the same solution (cold) for twenty-four hours; then immerse them first in a weak, then in a strong solution of chlorid of iron. The cover-glasses are washed in water (sections in alcohol) and subsequently passed through the usual reagents for dehydration and clearing.

By these methods of staining some syphilitic tissues are found to contain bacilli closely resembling the tubercle bacillus. They are about the same size, are distinctly curved, and often present club-like enlargements of one end (involution-forms?). The bacilli occur singly and in groups. They never lie free in the interspaces of the tissue, but are always inclosed in cells, in this respect resembling those of leprosy. The bacilli are not always to be found in syphilitic lesions, nor is their demonstration easy under the most favorable circumstances. Lustgarten emphasizes, particularly, that they are only demonstrable after the most painstaking technical procedures.

The probable specificity of Lustgarten's bacillus was lessened by the observation by Matterstock,† Alvarez,‡ and Tavel, that preputial and vulvar smegma taken from healthy individuals commonly contained an organism similar both in morphology and staining. The occurrence of Lustgarten's bacillus in lesions of the internal organs could not but argue against the probability of its identity with the smegma bacillus; but Lustgarten himself pointed

* Baumgarten's "Jahresberichte," 1885, p. 96.

† "Mitt. med. Klin. Würzburg," 1885.

‡ "Archiv de Physiol. normal et Path.," 1885.

out that the bacilli of both tuberculosis and leprosy stain by his method, and thus gave Baumgarten the right to suggest that the few cases well adapted for the demonstration of the Lustgarten bacilli in the internal organs might be cases of mixed infection of tuberculosis and syphilis. The organism is no longer considered to be specific for syphilis, and has fallen into oblivion.

Bacillus of van Niessen.*

General Characteristics.—A motile, flagellated, sporogenous, aerobic and optionally anaerobic, non-chromogenic bacillus cultivated from the blood of syphilitics, cultivable upon all the usual media, and capable of being stained by all methods, including that of Gram, but not resisting the decolorant effects of acids.

Van Niessen has cultivated a bacillus from the serum of syphilitics that he looks upon as specific. The researches of others, however, have, up to the present time, entirely failed to confirm van Niessen's results.

Spirochæta pallida (Schaudinn).

For a long time it has been known that in preputial smegma and in various ulcerative lesions of the generative organs, certain spiral organisms could be demonstrated. Bordet studied these with some care, expecting to prove that they were concerned with the etiology of syphilis. It remained, however, for Schaudinn and Hoffmann† to point out that there were two spiral organisms, one which he calls Spirochæta refringens, which is found in almost all ulcerative lesions of the genitalia, and another which he calls Spirochæta pallida, which is found only in primary and secondary syphilitic lesions. The latter organism is difficult to stain and the best results were obtained by Giemsa's or Marino's Azur Eosin.‡ The observations of Schaudinn and Hoffmann have been fully confirmed by Metschnikoff§ and it seems probable that Spirochæta pallida is of some etiologic importance in syphilis.

*"Centralbl. f. Bakt. u. Parasitenk.," Bd. xxiii, No. 2, Jan. 19, 1898, p. 49; Nos. 3 and 4, Jan 31, 1898, p. 97; and Nos. 5 and 6, Feb. 11, 1898, p. 177.

†"Deutsche med. Wochenschrift," May 4, 1905.

‡"Ann. de l'Inst.," 1904, p. 764.

§"Bull. Acad. de med. de Paris," May 16, 1905.

Protozoa and Syphilis.

Döhle * succeeded in staining certain protoplasmic bodies in the tissues in syphilis, which resembled bodies he had previously encountered in the discharges. They were for the most part round or oval, sometimes with irregular outline, and provided with flagella. They were stained in a mixture of hematoxylon and carbol-fuchsin, subsequently treated with iodin and washed in alcohol.

Convinced that these bodies were the cause of syphilis, he excised small fragments from gummata and other syphilitic tissues, and placed them beneath the skin of guinea-pigs, which subsequently fell ill with a chronic marasmus which ultimately caused death.

The work of Schüller† upon the protozoan parasite of syphilis is worth perusal.

* "Münchener med. Wochenschrift," 1897, No. 43.

† "Centralbl. f Bakt " etc Bd. xxxii, 1902, pp. 342, 433, 489, 609.

CHAPTER VI.

ACTINOMYCOSIS.

Actinomyces Bovis (Bollinger).

General Characteristics.—A parasitic, pathogenic, aerobic and optionally anaerobic, non-motile, non-flagellate, non-sporogenous (?), pathogenic branched micro-organism belonging to the higher bacteria, staining by ordinary methods and by Gram's method.

In 1845 Langenbeck discovered that an infectious disease of cattle known as "wooden tongue" and "lumpy jaw," and later as actinomycosis, could be communicated to man. The observation, however, was not published until 1878, one year after Bollinger* had discovered the *actinomyces*, the specific cause of the disease.

Israel † wrote the first important paper upon actinomycosis as a disease of man, though the best paper on the subject is probably that by Boström,‡ who made a careful study of the microscopic lesions of the disease.

Its first manifestations are usually found either about the jaw or in the tongue, and consist of considerable-sized enlargements which are sometimes dense and fibrous (wooden tongue), sometimes suppurative in character. In sections of tissue containing these nodular formations, small yellowish granules surrounded by some pus can usually be found. These granules, when examined beneath the microscope, consist of peculiar rosette-like bodies—the "ray-fungi" or *actinomyces*.

Distribution.—The actinomyces is known only as a parasitic organism associated with actinomycosis.

Morphology.—A complete ray-fungus consists of several distinct zones composed of different elements. The center is composed of a granular mass containing numerous bodies resembling micrococci or spores. Extending from this center into the neighboring tissue is a radiating, branched,

* "Deutsche Zeitschrift für Thiermedizin," 1877.

† "Virchow's Archives," 1874–1878.

‡ "Zeitschrift für Hygiene," 1889.

tangled mass of mycelial threads. In an outer zone these threads are seen to terminate in conspicuous, club-shaped, radiating forms which give the colonies their rosette-like appearance. The clubs are inconspicuous in the human lesions of the disease.

The pleomorphism of the organism and the branched network it forms class it among the higher bacteria in the genus *Actinomyces*. When the clumps formed in artificial cultivations of the parasite are properly crushed, spread

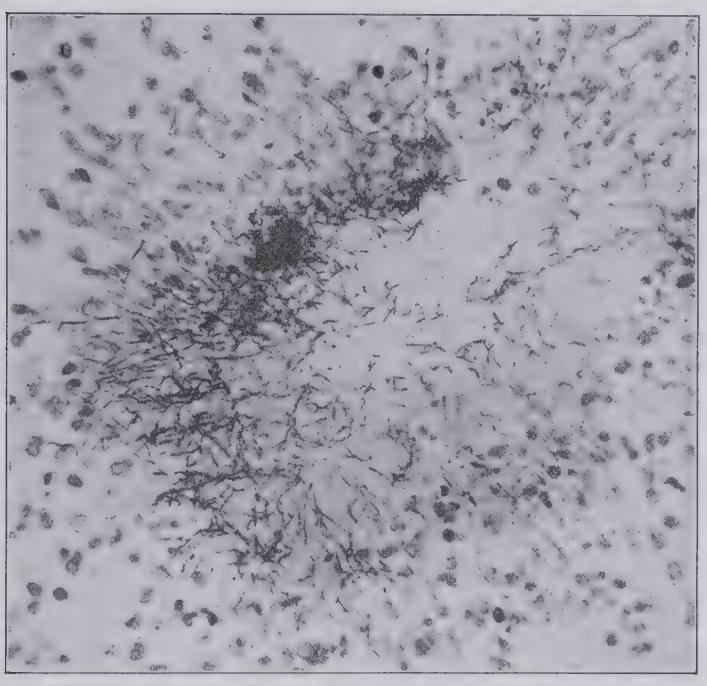


Fig. 108.—Colony or granule of actinomyces in a section through a lesion, showing the Gram-stained filaments and hyaline material and also the pus-cells surrounding the colony (Wright and Brown).

out, and stained, the long mycelial threads, 0.3–0.5 μ in thickness, frequently show flask- or bottle-like expansions —the clubs—at the ends. These probably depend upon gelatinization of the cell-membrane of the degenerating parasite. The club is one of the chief characteristics of the organism. In sections of tissue the radiating filaments are very distinct, and the terminal clubs are all directed outward, closely packed together, and making the whole mass form

a rounded little body often spoken of as an "actinomyces grain." When tissues are stained first with carmin and then by Gram's method, the fungous threads appear blue-black, the clubs red. The cells of the tissues affected and a larger or smaller collection of leukocytes form the surrounding resisting tissue-zone.

The fungus is of sufficient size to be detected in pus, etc., by the naked eye. It can be colored, in sections of tissue, by the use of Gram's or Weigert's stain. Tissues pre-stained with carmin, then by Weigert's method, show beautifully.

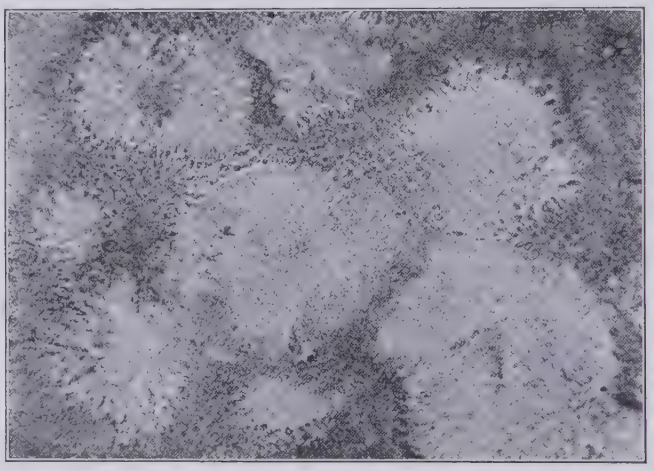


Fig. 109.—Actinomyces granule crushed beneath a cover-glass, showing radial striations in the hyaline masses. Preparation not stained; low magnifying power (Wright and Brown).

Cultivation.—The actinomyces fungus may be grown upon all the artificial culture media, as has been fully shown by Israel,* Wolff, and others.

To obtain a pure culture, material containing the actinomyces granules, secured so as to be as free as possible from contaminating micro-organisms, is crushed between glass plates or in a mortar, and the crushed fungi transferred to plates or tubes as desired. The colonies appear as small gray dots, and consist of a translucent, radiating filamentous network. If kept for a few days at 37° C. they become opaque and nodular, with radiating processes about the periphery. Still later they develop a whitish downy ap-

* "Virchow's Archives," cxv.

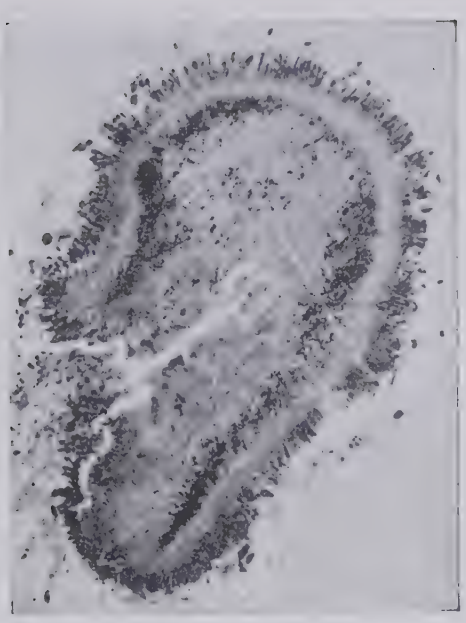


Fig. 110.—Colony of actinomyces with well-developed "clubs" at the periphery in a nodule in the peritoneal cavity of a guinea-pig inoculated with a culture from another guinea-pig. Paraffin section. Low magnification (Wright). (Photograph by Mr. L. S. Brown.)

Fig. 111.—A colony of actinomyces in a nodule twenty-eight days old in the peritoneal cavity of a guinea-pig inoculated with a culture from another guinea-pig (Bovine case). The "clubs" are well developed and show some indications of stratification. Paraffin section. × 750 aprox. (Wright). (Photograph by Mr. L. S. Brown.)

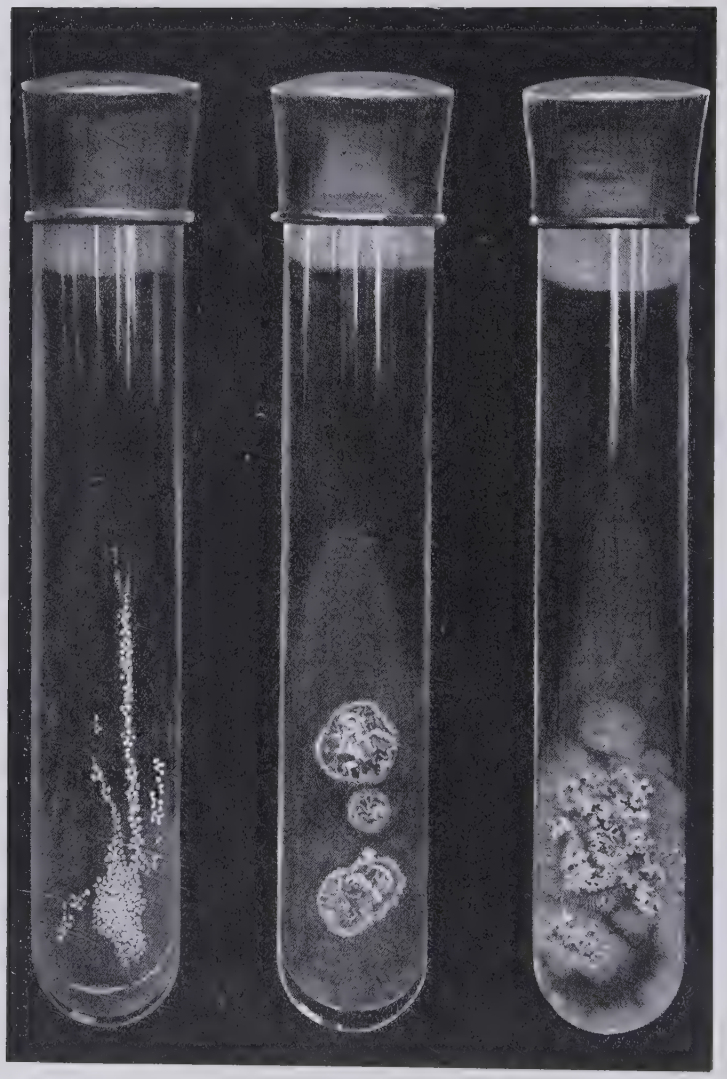


Fig. 112.—Actinomycosis; glycerin-agar cultures: *A*, Discrete rounded colonies after about ten days' incubation at 37° C.; *B*, limpet-shaped colonies three and a half months old; *C*, lichen-like appearance frequently seen; the growth is three and a half months old (Curtis).

pearance from the formation of short aerial hyphæ. The best growth occurs when free access of oxygen is permitted.

Blood-serum.—Upon blood-serum the nodular growths present a yellowish or rust-red color, and are surrounded with a whitish down of fine threads. The colonies adhere closely to the culture media and are so firm that they crush with difficulty. If the surface be scraped, spores and fine threads may be secured. If the mass be crushed, branched filaments may be secured. The colonies become confluent in the course of time, and a thick wrinkled membrane is produced. The growth liquefies blood-serum.

Gelatin.—In gelatin puncture cultures an arborescent growth occurs and the gelatin is liquefied.

Agar-agar.—Upon agar-agar and glycerin agar-agar the growth is similar to that upon blood-serum. The agar-agar turns brown as the culture ages.

Bouillon.—In bouillon the growth occurs in the form of large granules if allowed to stand quietly; of numerous small granules if frequently shaken up. The granules are similar in structure to those formed upon the dense media. The bouillon does not become clouded.

Potato.—Upon potato the growth resembles that upon blood-serum, but is slower in developing. The color is reddish-yellow and the white down early makes its appearance.

Eggs.—The organism can also be grown in raw eggs, into which it is carefully introduced through a small opening made under aseptic precautions. In the eggs long, branched mycelial threads quite unlike the bacillary forms that grow upon agar-agar are formed.

The characteristic rosettes so constantly found in the tissues are never seen in artificial cultures.

Virulence.—When the actinomyces is grown upon artificial media the virulence is retained for a considerable time.

Pathogenesis.—Actinomycosis is almost peculiar to bovine animals, but sometimes occurs in hogs, horses, and other animals, and rarely in human beings. The disease can with difficulty be inoculated into experiment animals, the introduced fungi either becoming absorbed or encapsulated by connective tissue and not growing. In the abdominal cavities of rabbits the peritoneum, mesentery, and omentum show typical nodules containing the actinomyces rays in cases of successful inoculation.

Mode of Infection.—The manner by which the organism enters the body is not positively known. In some cases

it may be by direct inoculation with infectious pus, but there is some reason to believe that the organism occurs in nature as a saprophyte, or as an epiphyte upon the hulls of certain grains, especially barley. Woodhead has recorded a case where a primary mediastinal actinomycosis in the

Fig. 113.—Section of liver from a case of actinomycosis in man (Crookshank).

human subject was apparently traced to perforation of the posterior pharyngeal wall by a barley spikelet accidentally swallowed by the patient.

Cases of actinomycosis are fortunately somewhat rare in

human medicine, and do not always occur in those brought in contact with the lower animals. The fungi may enter the organism through the mouth and pharynx, through the respiratory tract, through the digestive tract, or through wounds.

The invasion has been known to take place at the roots of carious teeth, and is more liable to occur in the lower than in the upper jaw. Israel reported a case in which the primary lesion seemed to occur external to the bone of the lower jaw, as a tumor about the size of a cherry, with an external opening. Two cases of the disease observed by Murphy, of Chicago, began with toothache and swelling of the jaw. A few cases of dermal infection are recorded. Elsching * has seen a case in which calcified actinomyces grains were observed in the tear duct.

When inhaled, the organisms enter the deeper portions of the lung and cause a suppurative broncho-pneumonia with adhesive inflammation of the contiguous pleura. After the formation of the pleuritic adhesions the disease may penetrate the newly formed tissue, extend to the chest-wall, and ultimately form external sinuses; or, it may penetrate the diaphragm and invade the abdominal organs, causing interesting and characteristic lesions in the liver and other large viscera (see Fig. 113).

Lesions.—The degree of chemotactic influence exerted by the organism seems to depend upon the tissue affected, upon the peculiarity of the animal, and upon the virulence of the organism. When an animal is but slightly susceptible, and especially when the tongue is affected, the disease is characterized by the formation of cicatricial tissue—"wooden tongue." If, on the other hand, the animal be highly susceptible and the jaw-bone affected, suppuration, with the formation of abscesses, osteoporotic cavities, and sinuses, are apt to be noticed. This form of the disease is called "lumpy jaw" in cattle.

Before the nature of the affection was understood it was confounded with diseases of the bones, especially osteosarcoma.

From the tissues primarily affected the disease spreads to the lymphatic glands, and eventually to the lungs. Israel has pointed out that certain cases of human actinomycosis begin in the peribronchial tissues, probably from inhalation of the fungi.

* "Centralbl. f. Bakt. u. Parasitenk.," XVIII, p. 7.

Microscopically the lesions consist chiefly of a round-cell infiltration with circumscribing granulation-tissue leading to the formation of cicatricial bands. In the form known as "wooden tongue" the disease runs an essentially chronic course, with the production of considerable amounts of connective tissue.

But few cases recover, the disease terminating in death from exhaustion or from complicating pneumonia or other organic lesions.

CHAPTER VII.

MYCETOMA, OR MADURA-FOOT.

Actinomyces Maduræ.

General Characteristics.—A non-motile, non-flagellate, sporogenous (?), non-liquefying, chromogenic, aerobic and optionally anaerobic, branched, parasitic organism belonging to the higher bacteria, staining by ordinary methods and by Gram's method, and pathogenic for man.

A curious disease of not infrequent occurrence in the Indian province of Scinde and of rare occurrence in other countries is known as mycetoma, Madura-foot, or *pied de Madura*. Although described as peculiar to Scinde, the disease is not limited to that province, but has been met with in Madura, Hissar, Bicanir, Delhi, Bombay, Baratpur, Morocco, Algeria, and in Italy. In America less than a dozen cases of the disease have been placed on record. In India it almost invariably affects natives of the agricultural class, and in nearly all cases is referred by the patient to the prick of a thorn. It usually affects the foot, more rarely the hand, and in one instance was seen by Boyce to affect the shoulder and hip. It is more common in men than in women, individuals between twenty and forty years of age suffering most frequently, though persons of any age may suffer from the disease. It is insidious in onset, no symptoms being observed in what might be called the incubation stage, which is of a couple of weeks' duration, but a nodular growth forming in the course of time and gradually attaining the size of a marble. Its deep attachments are indistinct and diffuse. The skin over it becomes purplish, thickened, indurated, and adherent. The ball of the great toe and the pads of the fingers and toes are the points most frequently invaded. Although very slowly, the lesions progress very perceptibly, and in the course of a few months form distinct inflammatory nodes. After a year or two the nodes begin to soften, break down, discharge necrotic and purulent material, occasioning the formation of ulcers

and sinuses. The matter discharged from the lesions at this stage of the disease is a thin sero-pus, and contains occasional fine round pink or black bodies, similar to actinomyces "grains," described, when pink, as resembling fish-roe; when black, as resembling gunpowder. It is upon the detection of these particles that the diagnosis rests. According to the color of the bodies found, cases are divided into the *pale* and *melanoid* varieties.

The progress of the disease causes an enormous enlargement of the affected part. The malady is usually painless.

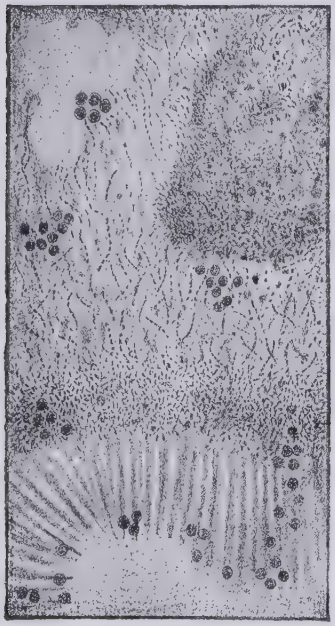


Fig. 114.—Streptothrix Maduræ in a section of diseased tissue (Vincent).

The micro-organismal nature of the disease was early suspected. In spite of the confusion caused by some who confounded the disease with "guinea-worm," Carter held that it was due to some indigenous fungus as early as 1874. Boyce and Surveyor found that the black particles of the melanoid variety consisted of a large branching septate fungus.

Pale Variety. — Kanthack was the first to prove the identity of the fungus with the well-known actinomyces, but there seems to be considerable doubt about the identity of the species.

Morphology.—Under the microscope the organism is found by Vincent* to be branched and belong to the higher bacteria. It consists of long, branched bacillary threads forming a tangled mass. In many of the threads spores could be made out. He was unable to communicate the disease to animals by inoculation.

Cultivation.—Vincent succeeded in isolating the specific micro-organism by puncturing one of the nodes with a

* "Ann. de l'Inst. Pasteur," 94, 3.

sterile pipet, and cultivated it upon artificial media, acid vegetable infusions seeming best adapted to its growth. It develops scantily at the room temperature, better at 37° C.—in from four to five days. In twenty to thirty days a colony attains the size of a little pea.

Bouillon.—In bouillon and other liquid media the organisms form little clumps resembling those of actinomyces. They cling to the glass, thus remain near the surface of the medium, and develop a rose- or bright-red color. Those which sink to the bottom form spheric balls devoid of the color.

Gelatin.—The growth in gelatin is not very abundant, and forms dense, slightly reddish, rounded clumps. Sometimes there is no color. There is no liquefaction.

Agar-agar.—Upon the surface of agar-agar beautiful rounded, glazed colonies are formed. They are at first colorless, but later become rose-colored or bright red. The majority of the clusters remain isolated, some of them attaining the size of a small pea. They are usually umbilicated like a variola pustule, and present a curious appearance when the central part is pale and the periphery red. As the colony ages the red color is lost and the colony becomes dull white or downy from the formation of aerial hyphæ. The colonies are very adherent to the surface of the medium, and are almost of cartilaginous consistence.

Milk.—The organism grows in milk without causing coagulation.

Potato.—Upon potato the growth of the organism is meager and slow, with very little chromogenesis. The color-production is more marked if the potato be acid in reaction. Some of the colonies upon agar-agar and potato have a powdery surface, either from the formation of spores or of aerial hyphæ.

Lesions.—Microscopic study of the diseased tissues in mycetoma is not without interest. The healthy tissue is sharply separated from the diseased areas, which appear like large degenerated tubercles, except that they are extremely vascular. The mycelial or filamentous mass occupies the center of an area of degeneration, where it can be beautifully demonstrated by the use of appropriate stains, Gram's and Weigert's methods being excellent for the purpose. The tissue surrounding the nodes is infiltrated with small round cells. The youngest nodules consist of

granulation-tissue, whose development is checked by early coagulation-necrosis. Giant-cells are few.

Not infrequently small hemorrhages occur from the ulcers and sinuses of the diseased tissues; the hemorrhages can be explained by the abundance of small blood-vessels in the diseased tissue.

The Melanoid Form of mycetoma has been carefully investigated by Wright * and appears to depend upon an entirely different micro-organism properly classed among the hyphomycetes. It is probably identical with the organism described by Boyce and Surveyor.

In the case studied, Wright found the diseased tissues, consisting of several of the pads of the toes, to be either translucent and myxomatous or yellowish and necrotic in appearance. The black granules were embedded in the tissue and appeared mulberry-like and less than 1 mm. in diameter. They were firm, and when enucleated and pressed between cover and slide did not crush. Only after digestion with a solution of caustic potash and careful teasing could the granules be resolved into the hyphæ of the mold. The central part of the granule formed a reticulum, with radiating, somewhat clavate elements projecting from it.

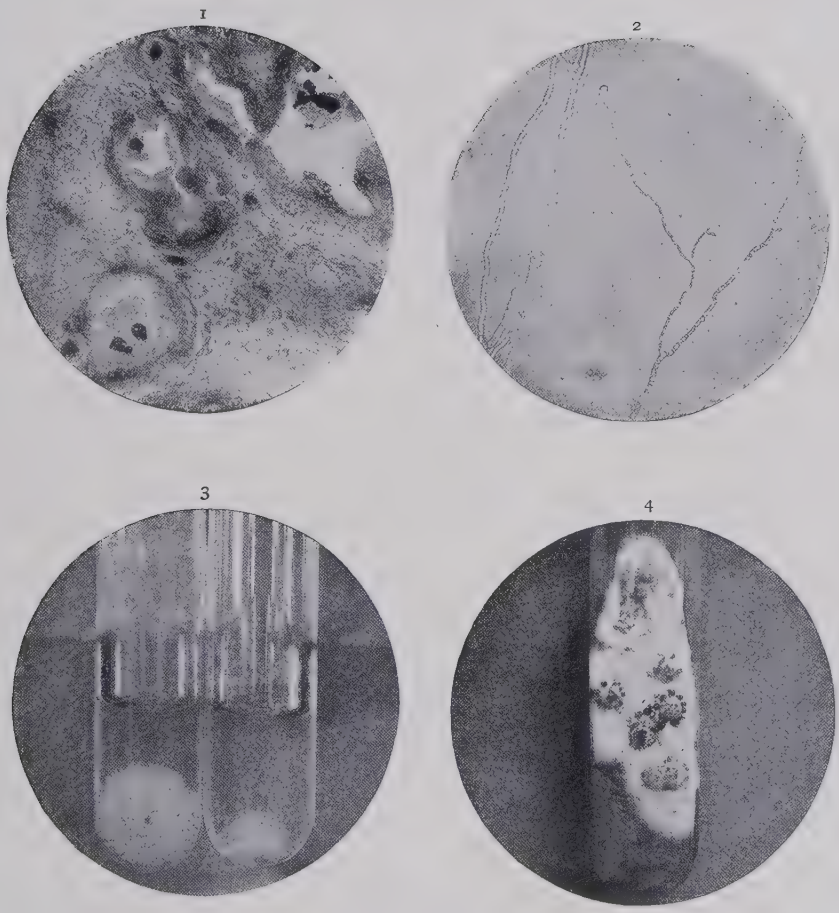
In sections of tissue it was found possible to stain the fungus with Gram's and Weigert's stains, though prolonged washing removed most of the dye.

Cultural Characteristics.—Enucleated granules carefully washed in sterile bouillon and then planted upon agar-agar afforded cultures of the mold in 25 out of 65 attempts.

The growth began in five or six days, appearing on solid media as a tuft of delicate whitish filaments, springing from the black grain, and in a few days covering the entire surface of the medium with a whitish or pale brown felt-work. Upon potato this felt-work supports drops of brownish fluid. The long branched hyphæ thus formed were from 3 to 8 μ in diameter, with transverse septa in the younger ones. The older hyphæ were swollen at the ends. No buds were observed. No fruit organs were detected. In fluid media the filaments radiated from the central grain with the formation of a kind of puff-ball. Eventually the whole

* "Journal of Experimental Medicine," vol. III, 1898, p. 421.

PLATE II.



MELANOID FORM OF MYCETOMA.—1. Section showing black granules and general features of the lesions as they appear under a low magnifying power. Zeiss a_2. 2. Showing structure and appearances of the hyphæ of the mycelium obtained from the granules. Zeiss apochromat., 4 mm. 3. Two bouillon cultures, showing the powder-puff-ball appearance. In one the black granule is seen in the center of the growth. 4. Potato culture of the hyphomycete obtained from the granules. The black globules are composed of a dark-brown fluid. (James H. Wright.)

medium becomes filled with mycelia and a definite surface growth forms.

The general characteristics of the fungus are well shown in the accompanying illustrations from Wright's paper (Figs. 110, 111).

CHAPTER VIII.

FARCIN DU BOEUF.

Actinomyces Farcinica.

General Characteristics.—A parasitic, pathogenic, aerobic and optionally anaerobic, non-motile, non-flagellate, sporogenous (?), non-liquefying, branched organism pathogenic for cattle, belonging to the higher bacteria, and staining by Gram's method.

A peculiar disease of cattle common in Guadeloupe and occasionally observed elsewhere was described by the older writers as *farcin du bœuf*. It is characterized by a superficial lymphangitis and lymphadenitis, extending to the tracheal, axillary, prescapular, and other glands. The affected glands enlarge, suppurate, and discharge a creamy, sometimes a grumous, pus. The internal organs are often affected with a resulting pseudo-tuberculosis whose central areas undergo purulent or caseous degeneration.

Nocard* found that after staining with Gram's and Kühne's methods peculiar micro-organisms could be defined in the centers of the tubercles. These he described as Bacillus du Farcin. Trevisan, in 1889, recognizing that they were not bacilli, created a new genus for them and described the organisms as Nocardia farcinica. In 1891 Rossi-Doria † included it among the streptothrices, calling it Streptothrix farcinica. According to the nomenclature adopted by the author, it appears to be more correctly described as an actinomyces than as a streptothrix.

Morphology.—The organisms consist of long delicate filaments intricately woven, and are characterized by distinct branching, which make clear their proper classification among the actinomycetes. The organism was successfully cultivated by Nocard upon various culture media at the temperature of the body. It is aerobic.

Microscopic study of the cultures reveals the same mass of tangled branched filaments seen in the tissues. Old

* "Ann. de l'Inst. Pasteur," 1888, t. II, p. 293.

† "Annali de l'Instituto d'igiene sperimentale di Roma," 91.

cultures are rich in spores, which are very small and develop upon the most superficial portions only.

Cultivation.—Bouillon.—In bouillon the organism develops in the form of colorless masses irregular in size and shape, some of which float upon the surface, while others sink to the bottom of the liquid. Sometimes the surface is covered by an irregular fenestrated pellicle of a gray color.

Agar-agar.—Upon agar-agar (glycerin agar-agar is preferable) the growth develops in the form of small, discrete, irregularly rounded, opaque masses of a yellowish-white color. The surface of the colonies is irregular and uneven, the appearance resembling a lichen (see Fig. 115).

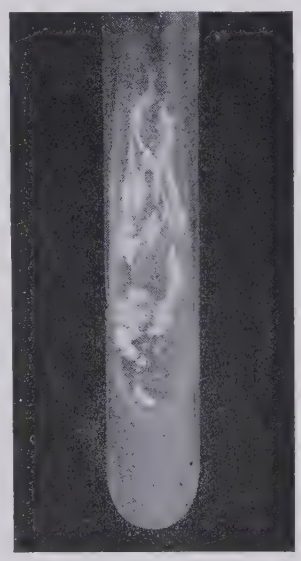


Fig. 115.—Streptothrix of farcin du bœuf growing on glycerin agar-agar.

Potato.—Upon potato a pale yellow, dry, wrinkled, scaly growth occurs.

Blood-serum.—The growth upon blood-serum is similar to that upon agar-agar, but is less luxuriant.

Milk.—In milk the organism produces no coagulation and does not alter the reaction.

Pathogenesis.—The micro-organism of *farcin du bœuf* is pathogenic for guinea-pigs, cattle, and sheep. Man, dogs, rabbits, horses, and asses are immune.

When a virulent culture, or some pus, containing the micro-organisms, is subcutaneously injected into a guinea-pig, an abscess forms at the seat of operation. Not long afterward the lymphatic vessels and glands of the region swell and indurate, and extensive phlegmons form, which rupture externally and discharge pus. The animal becomes extremely ill, seems about to die, but slowly recovers.

In the cow and the sheep subcutaneous inoculations result in the formation of an abscess relatively less extensive, which evacuates, ulcerates, indurates, and seems to

disappear, but which, after the lapse of several weeks or months, again opens and forms a new abscess.

In animals which are immune or nearly immune, like the horse, the ass, the dog, and the rabbit, the subcutaneous inoculation is followed by the formation of a small abscess which speedily cicatrizes.

Intraperitoneal inoculation in the guinea-pig gives rise to lesions resembling tubercles. The omentum may be extensively involved and full of softened nodes, and the liver, spleen, and kidneys appear full of tubercles, but careful examination will show that the tubercles occur only upon the peritoneal surfaces, not in the organs.

Intravenous introduction of the cultures produces a condition much resembling general miliary tuberculosis. All the organs contain the pseudo-tubercles in considerable numbers.

Virulence.—Nocard found a culture still virulent after it had been kept for four months in an incubating oven at 40° C.

CHAPTER IX.

RHINOSCLEROMA.

Bacillus Rhinoscleromatis (von Frisch*).

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-chromogenic, aerobic and optionally anaerobic, capsule bacillus, pathogenic for man and identical with Bacillus pneumoniæ of Friedländer, except that it does not stain by Gram's method.

A peculiar disease of the nares, characterized by the formation of circumscribed nodular tumors, and known as *rhinoscleroma*, is occasionally seen in Austria-Hungary, Italy, and some parts of Germany. A few cases have been observed among the foreign-born residents of the United States. The nodular masses are flattened, may be discrete, isolated, or coalescent, grow with great slowness, and recur if excised. The disease commences in the mucous membrane and the adjoining skin of the nose, and spreads to the skin in the immediate neighborhood by a slow invasion, involving the upper lip, jaw, hard palate, and sometimes even the pharynx. The growths are without evidences of acute inflammation, do not ulcerate, and upon microscopic examination consist of an infiltration of the papillary layer and corium of the skin, with round cells which in part change to fibrillar tissue. The tumors possess a well-developed lymph-vascular system. Sometimes the cells undergo hyaline degeneration.

In the nodes von Frisch discovered bacilli closely resembling the pneumobacillus of Friedländer, both in morphology and vegetation, and, like it, surrounded by a capsule. The only differences between the bacillus of rhinoscleroma and Bacillus pneumoniæ of Friedländer are that the former stains well by Gram's method, while the latter does not; that the former is rather more distinctly rod-shaped than the latter, and more often shows its capsule in culture media.

The bacillus can be cultivated, and cultures in all media

* "Wiener med. Wochenschrift," 1882, 32.

resemble those of the bacillus of Friedländer (*q. v.*) so closely as to be indistinguishable from it. Even when inoculated into animals the bacillus behaves much like Friedländer's bacillus.

Inoculation has, so far, failed to reproduce the disease either in man or in the lower animals.

(B) THE TOXEMIAS.

CHAPTER I.

TETANUS.

Bacillus Tetani (Flügge).

General Characteristics.—A motile, flagellated, sporogenous, liquefying, obligatory anaerobic, non-chromogenic, toxic, pathogenic bacillus of the soil, staining by ordinary methods and by Gram's method. Its chief morphologic characteristic is the occurrence of a large round spore at one end.

The bacillus of tetanus was discovered by Nicolaier * in 1884, and obtained in pure culture by Kitasato † in 1889. It is universally acknowledged to be the cause of tetanus.

Distribution.—The tetanus bacillus is a common saprophyte in garden earth, dust, and manure, and is a constant parasite in the intestinal canal of herbivorous animals.

The relation of the bacillus to manure is interesting, but it is most probable that manured ground, because it is richer, permits the bacilli to flourish better than sterile ground. The common occurrence of the bacilli in the excrement of herbivorous animals is to be explained through the accidental ingestion of earth with the food cropped from the ground. The spores of the bacillus thus reaching the intestine, seem able to develop because of appropriate anaerobic conditions.

Verneuil has observed that tetanus rarely occurs at sea except upon cattle transports, where there is likely to be considerable earth and dust from hay, straw, etc., which may carry the bacilli.

Le Dantec ‡ has shown that the tetanus bacillus is a

* "Deutsche med. Wochenschrift," 1884, 42.

† *Ibid.*, 1889, No. 31.

‡ See abstracts in the "Centralbl. f. Bakt. u. Parasitenk.," IX, 286; XIII, 351.

common organism in New Hebrides, where the natives poison their arrows by dipping them into a clay rich in tetanus bacilli.

Morphology.—The tetanus bacillus is a long, slender organism measuring 0.3–0.5 × 2–4 μ (Flügge). Its most striking characteristic is an enlargement of one end, which contains a large round spore (Fig. 116). The bacilli in which no spores are yet formed have rounded ends and seldom unite in chains or pairs. They are motile, and have many flagella arising from all parts of the surface (peritricha).

Staining.—The bacilli stain readily with ordinary aqueous solutions of the anilin dyes and by Gram's method.

Fig. 116.—Bacillus tetani. × 1000 (Fränkel and Pfeiffer).

Isolation.—The method usually employed for the isolation of the tetanus bacillus was originated by Kitasato, and based upon the observation that its spores can resist exposure to high temperatures for considerable periods of time. After finding by microscopic examination that the bacilli were present in pus, Kitasato spread it upon the surface of an ordinary agar-agar tube and incubated it for twenty-four hours, during which time all of the contained micro-organisms, including the tetanus bacillus, increased in number. He then exposed it for an hour to a temperature of 80° C., by which all fully developed bacteria, tetanus as well as the

others, and the great majority of the spores, were destroyed. As scarcely anything but the tetanus spores remained alive, their subsequent growth gave a fairly pure culture.

Cultivation.—The tetanus bacillus is difficult to cultivate, because it will not grow where the smallest amount of free oxygen is present. It is hence a typical obligatory

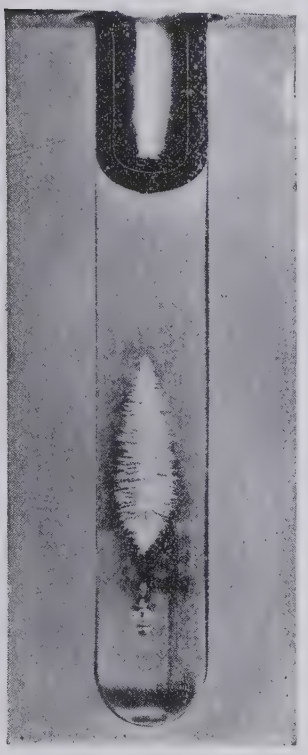


Fig. 117.—Bacillus tetani; six-days-old puncture culture in glucose-gelatin (Fränkel and Pfeiffer).

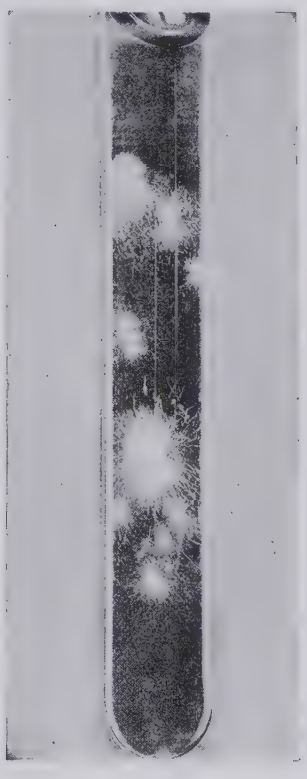


Fig. 118.—Bacillus tetani; culture four days old in glucose-gelatin (Fränkel and Pfeiffer).

anaerobe. Farran * and Grixoni believe it to have originally been an optional anaerobe, and it is said by these writers that the organism can gradually be accustomed to oxygen so as to grow in its presence. When this is achieved, it loses its virulence.

* "Centralbl. f. Bakt. u. Parasitenk.," July 15, 1898, p. 28.

The methods for excluding the oxygen from the cultures and replacing it by hydrogen, as well as other methods suggested for the cultivation of the strictly anaerobic organisms, are given under the appropriate heading (Anaerobic Cultures), and need not be repeated here.

I have suggested a simple method of cultivating the bacillus in bouillon for the purpose of securing its toxin * in an ordinary bottle which is filled to the mouth with bouillon, then closed by a perforated rubber stopper containing a glass tube a couple of inches long. Connected with this glass tube, by means of a short piece of rubber tubing (*a*), is the bulb of a broken pipet (*b*), the other end of which is plugged with cotton (Fig. 119). The only object of this attachment is to provide for the expansion of the contained fluid during steam sterilization. When the steam sterilization takes place, the expanding fluid ascends to the reservoir represented by the pipet bulb (*b*), descending again as the fluid cools. When the sterilization is completed, the reservoir is detached at *a*, the inoculation of the bouillon made by passing a fine pipet through the glass tube into the bottle, the projecting glass tube heated in a flame and drawn out to a fine tube. The bottle is then stood in hot water until the expanding fluid ascends to the top of the glass tube, when it is sealed in a flame and the bottle and its contents permitted to cool. In cooling, the retracting fluid leaves a partial vacuum which at once draws up any minute bubbles of air remaining, and allows the tetanus bacillus to grow in a condition of almost perfect anaerobiosis.

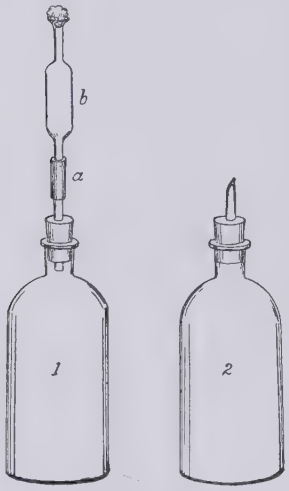


Fig. 119.—Tetanus bottle: 1, During sterilization; 2, after inoculation and sealing.

Park,† following the suggestion of Kitasato, covers the surface of the bouillon with a layer of paraffin about 1–2 cm.

* "Centralbl. f. Bakt. u. Parasitenk.," XIX, Nos. 14 and 15, April 25, 1896, p. 550.

† "Jour. Med. Research," N. S., vol. 1, No. 1, p. 298.

thick. This melts in the sterilization and forms a firm layer, through which the bouillon is inoculated, warmed until the paraffin melts again, then stood away until development in the air-free bouillon occurs. If the paraffin be found too brittle, some albaline may be mixed with it until it is flexible when cool.

The **colonies** of the tetanus bacillus, when grown upon gelatin plates, in an atmosphere of hydrogen resemble those of the well-known hay bacillus. There is a rather dense, opaque central mass surrounded by a more transparent

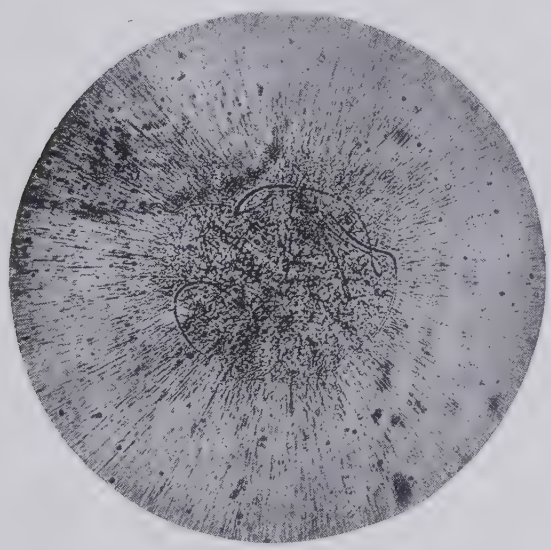


Fig. 120.—Bacillus tetani; five-days-old colony upon gelatin containing glucose. × 1000 (Fränkel and Pfeiffer).

zone, the margins of which consist of a fringe of radially projecting bacilli (Fig. 120). Liquefaction occurs slowly.

Bouillon.—The organism can be grown in bouillon, and attains its maximum development at a temperature of 37° C. Gas is given off from the cultures, and they have a peculiar odor, very characteristic, but difficult to describe. The bouillon is clouded and contains a sediment.

Gelatin.—The growth occurs deep in the puncture, and is arborescent (Fig. 121). Liquefaction begins in the second week and causes the disappearance of the radiating filaments. The liquefaction spreads slowly, but may involve the en-

tire mass of gelatin and resolve it into a grayish-white syrupy liquid, at the bottom of which the bacilli accumulate. The growth in gelatin containing glucose is rapid.

Fig. 121. — Tetanus bacillus; glucose-agar culture, five months old (Curtis).

Agar-agar.—The growth in agar-agar punctures is slower, but similar to the gelatin cultures except for the absence of liquefaction.

Vital Resistance.—The tetanus spores may remain alive in dry earth for many years. Sternberg says they can resist immersion in 5 per cent. aqueous carbolic acid solutions for ten hours, but fail to grow after fifteen hours. A 5 per cent. carbolic acid solution, to which 0.5 per cent. of hydrochloric acid has been added, destroys them in two hours. They are destroyed in three hours by 1:1000 bichlorid of mercury solution; but when to such a solution 0.5 per cent. of hydrochloric acid is added, its activity is so increased that the spores are destroyed in thirty minutes. Exposure to streaming steam for from five to eight minutes is certain to kill them.

Toxic Products.—Bouillon cultures of the tetanus bacillus contain a powerful toxin in solution.

The most ready method of preparing it for experimental study is to cultivate the bacilli in bouillon, at a temperature of 37° C., and then filter the culture through porcelain. Field* found the highest degree of toxicity about the sixth or seventh day. It may attain a toxicity so great that 0.000005 c.c. will cause the death of a mouse. I found the average toxicity such that 0.001 c.c. was fatal to a guinea-pig. The toxin is very unstable and is easily de-

*"Proc. N. Y. Path. Soc.," Mar., 1904, p. 18.

stroyed by heat above 60°–65° C. It is also decomposed by exposure to the air and light, so that it is difficult to preserve it for many days. The best method of keeping it is to add 0.5 per cent. of ph nol, and then store it in a cool, dark place, in bottles completely filled and tightly corked. It will not keep its strength in liquid form under the best conditions.

To keep it for experimental purposes it is advisable to precipitate it by supersaturation with ammonium sulphate, which causes it to float in the form of a sticky brown scum upon the liquid. It can be skimmed off and dried. Such dry precipitate will retain its activity for months with but little deterioration.

Fermi and Pernossi * found most toxin produced in agar-agar cultures, less in gelatin cultures, and least in bouillon cultures.

Ehrlich † found two poisons in the tetanus toxin, one of which was convulsive and was in consequence called *tetanospasmin*, the other hemolytic and called *tetanolysin*. When tetanus toxin is added to defibrinated blood, the tetanolysin is absorbed by the corpuscles, many of which are dissolved, while the tetanospasmin remains unchanged.

Dönitz ‡ and Wassermann and Takaki § have found that the tetanus toxin has a specific affinity for the central nervous system, with whose cells it combines *in vitro* and becomes inert.

Roux and Borrel ‖ have also found that when tetanus toxin is injected into the brain substance a very much smaller dose will cause death than is necessary when the poison is absorbed from the subcutaneous tissues.

From cultures of tetanus bacilli grown in various media, and from the blood and tissues of animals affected with the disease, Brieger succeeded in separating "tetanin," "tetanotoxin," " tetanospasmin," and a fourth substance to which no name is given. All were very poisonous and productive of tonic convulsions. Later Brieger and Fränkel isolated an extremely poisonous toxalbumin from sugar-bouillon cultures of the bacillus.

The purified toxin of Brieger and Cohn was surely fatal

* "Centralbl. f. Bakt.," etc., xv, p. 303.
† "Berliner klin. Wochenschrift," 1898, p. 273.
‡ "Deutsche med. Wochenschrift," 1897, p. 428.
§ "Berliner klin. Wochenschrift," 1898, 35.
‖ "Ann. de l'Inst. Pasteur," t. xii, 1898.

to mice in doses of 0.00000005 gram. Lambert * considers the tetanus toxin to be the most poisonous substance that has ever been discovered.

Like most of the bacterial toxins, the tetanus poison is only effective when produced in or injected into the tissues and absorbed into the circulation. It is harmless when given by the digestive tract, Ramon † having administered by the mouth 300,000 times the fatal hypodermic dose without producing any symptoms. The toxin seemed to pass out with the feces.

The local action of the toxin is very painful and associated with spasm of the muscular fibers with which it comes in contact. Pitfield,‡ thinking that it might be useful in the treatment of certain paralytic affections, injected a minute quantity of it into the calf of his leg and experienced the severe spasmodic local effects of the poison for twelve hours.

It has been the belief of most physiologists that tetanus toxin acts solely upon the motor cells of the spinal cord, and produced the tonic spasms as strychnia does. Zupnik § has brought forward evidence that this view is incorrect and that there are two distinct actions caused by the toxin. He differentiates between *tetanus ascendens* and *tetanus descendens*. The former always succeeds the intramuscular introduction of the toxin, and depends upon its direct action upon the muscle itself. It explains the familiar phenomenon of rigidity making its first appearance in that member into which the inoculation was made. The ascending tetanus gradually ascends from muscle to muscle. He thinks the absorption of the poison by the muscle-cells depends upon their normal metabolic function, as when their nerves are severed, the fixation of the toxin and the occurrence of the tonic spasm does not occur.

Tetanus descendens results from the entrance of the toxin into the circulation from the cellular tissue and its distribution in the blood. Under these conditions Zupnik believes it acts upon the central nervous system, especially upon the spinal cord, manifesting itself in extreme reflex excitability with irregular motor discharges resulting in clonic spasms.

* "New York Med. Jour.," June 5, 1897.

† "Deutsche med. Wochenschrift," Feb. 24, 1898

‡ "Therapeutic Gazette," March 15, 1897.

§ "Wiener klin. Wochenschrift," Jan. 23, 1902.

There are, therefore, two forms of spasm in tetanus: the *tonic* convulsions, seeming to depend upon local action and fixation of the toxin, and the *clonic* convulsions, depending upon the centric action. The latter are the more dangerous for the sufferer.

The lockjaw or *trismus* and the *opisthotonos* that are so characteristic of the affection depend, according to Zupnik's view, upon a loss of equilibrium among the muscles affected. They occur only in descending tetanus and depend upon spasm of muscles without equally powerful opposing groups. The stronger muscles of the jaw are those that close it; the stronger muscles of the back, those of the erector group. This view is exactly the opposite of Meyer and Ransom,* who believe that the tetanus toxin is absorbed only along the nerve trunks, and found that section of the spinal cord prevented the ascent of tetanus from the lower extremities. Injection of the toxin into a posterior nerve-root produced tetanus dolorosus. Injection of the toxin into a posterior nerve-root together with section of the spinal cord produced exaltation of the reflex irritability—"Jactationstetanus." Injection in sensory nerves does not produce tetanus dolorosus because the transportation of the poison along these trunks is so slow.

Pathogenesis.—The work of Kitasato has given us very complete knowledge of the biology of the tetanus bacillus and completely established its specific nature.

When a white mouse is inoculated with an almost infinitesimal amount of tetanus culture, or with garden earth containing the tetanus bacillus, the first symptoms come on in from one to two days, when the mouse develops typical tetanic convulsions, first beginning in the neighborhood of the inoculation but soon becoming general. Death follows sometimes in a very few hours. In rabbits, guinea-pigs, mice, rats, and other small animals the period of incubation is from one to three days. In man the period of incubation varies from a few days to several weeks. It averages about nine days.

The disease is of much interest because of its purely toxic nature. There is usually a small wound with a slight amount of suppuration and at the autopsy the organs of the body are normal in appearance, except the nervous system,

*"Archiv. f. experimentelle Path. u. Pharmak.," Bd. XLIX, 1903, p. 369.

which bears the greatest insult. It, however, shows little else than congestion either macroscopically or microscopically.

The conditions in the animal body are in general unfavorable to the development of the bacilli, because of the loosely combined oxygen contained in the blood, and they grow with great slowness, remaining localized at the seat of inoculation, and never entering the blood. Doubtless most cases of tetanus are cases of mixed infection in which the bacillus enters with aerobic bacteria, which aid its growth by absorbing the oxygen in the neighborhood. The amount of poison produced must be exceedingly small and its power tremendous, else so few bacilli growing under adverse conditions could not produce fatal toxemia. The toxin is produced rapidly, for Kitasato found that if mice were inoculated at the root of the tail, and the skin and the subcutaneous tissues around the inoculation afterward either excised or burned out, the treatment would not save the animal unless the operation were performed *within an hour after the inoculation.*

Some incline to the view that the toxin is a ferment, and the experiments of Nocard* might be adduced in support of the theory. He says: "Take three sheep with normal tails, and insert under the skin at the end of each tail a splinter of wood covered with the dried spores of the tetanus bacillus; watch these animals carefully for the first symptoms of tetanus, then amputate the tails of two of them 20 cm. above the point of inoculation, . . . the three animals succumb to the disease without showing any sensible difference."

The circulating blood of diseased animals is fatal to susceptible animals because of the toxin which it contains; and the fact that the urine is also toxic to mice proves that the toxin is excreted by the kidneys.

The organisms usually enter the body through a wound caused by some implement which has been in contact with the soil, or enter abrasions from the soil directly. Doubtless many of the wounds are so small that their existence is overlooked, and this, together with the fact that the period of incubation of the disease, especially in man, is of considerable duration (three to nine days), and at times permits the wound to heal before any symptoms of intoxication

*Quoted before the Académie de Médicine, Oct. 22, 1895.

occur, serves to explain the occurrence of some of the reported cases in which no wound is said to have existed.

There are two classes of infected wounds particularly prone to be followed by tetanus—namely, those into which soil has been carried by the injuring implement and those of considerable depth. The infecting organism reaches the first class in large numbers, but finds itself under aerobic and other inappropriate conditions of growth. It reaches the second class in smaller numbers, but finds the conditions of growth better because of the depth of the wound.

The severity of the wound has nothing whatever to do with the occurrence of tetanus, pin-pricks, nail punctures, insect stings, vaccination, and a variety of other mild injuries sometimes being followed by it.

An interesting fact has been presented by Vaillard and Rouget,* who found that if the tetanus spores were introduced into the body freed from their poison, they were unable to produce the disease because of the promptness with which the phagocytes took them up. If, however, the toxin was not removed, or if the body-cells were injured by the simultaneous introduction of lactic acid or other chemic agent, the spores would immediately develop into bacilli, begin to manufacture toxin, and produce the disease. This suggests that many wounds may be infected by the tetanus bacillus though the surrounding conditions rarely enable it to develop satisfactorily and produce enough toxin to cause disease.

In very rare cases tetanus may possibly occur without the previous existence of a wound, as in the case reported by Kamen, who found the intestine of a person dead of the disease rich in Bacillus tetani. Kamen is of the opinion that the bacilli can grow in the intestine and be absorbed, especially where imperfections in the mucosa exist. It is not impossible, though he does not think it probable, that the bacteria growing in the intestine can elaborate enough toxin to produce the disease by absorption.

A peculiar observation has been made by Montesano and Montesson,† who unexpectedly found the tetanus bacillus in pure culture in the cerebro-spinal fluid of a case of paralytic dementia that died without a tetanic symptom.

* See "Centralbl. f. Bakt., Infekt., u. Parasitenk.," vol. xvi, p. 208.

† "Centralbl. f. Bakt. u. Parasitenk.," Dec., 1897, Bd. xxii, Nos. 22, 23, p. 663.

Immunity.—All animals are not alike susceptible to tetanus. Men, horses, mice, rabbits, and guinea-pigs are susceptible; dogs much less so. Cattle suffer chiefly after accouchement, and after abortion. Most birds are scarcely at all susceptible either to the bacilli or to their toxin. Amphibians and reptiles are immune, though it is said that frogs can be made susceptible by elevation of their body-temperature.

Antitoxin.—By the gradual introduction of tetanus toxin Behring and Kitasato * have been able to produce a powerful antitoxic substance in the blood of animals.

The method of obtaining *tetanus antitoxic serum* is much like that employed for securing diphtheria antitoxic serum (*q. v.*), except that a longer time is required for immunizing the animals, and that the doses of toxin administered are of necessity smaller because of its greater activity. Horses, dogs, and goats may be used for the purpose.

Madsen † found that for each of the specific poisons, tetanolysin and tetanospasmin, a specific antitoxin is produced, the one annulling the convulsive, the other the hemolytic properties of the toxin.

As tetanus is not a common affection, and the antitoxic serum, like most immune serums, is not permanent, Tizzoni and Cattani,‡ who have experimented extensively upon the subject, have successfully prepared it in a solid form, in which, it is claimed, it can be kept indefinitely, shipped any distance, and used after simple solution in water. The method consists in precipitating the antitoxin from the blood of immunized dogs with alcohol.

The strength of the serum is usually expressed 1 : 1,000,000, 1 : 10,000,000, etc., which indicates that 1 c.c. of the serum is capable of protecting 1,000,000 or 10,000,000 grams of guinea-pig from infection.

Behring and Knorr § have introduced an arbitrary unit by which antitoxic strength of the tetanus serum can be expressed. It is difficult to define in plain terms, but seems to be *the least quantity of the serum that will protect a 250-gram guinea-pig against 1,000,000 fatal doses of fresh toxin.*

* "Deutsche med. Wochenschrift," 1890, No. 49.

† "Zeitschrift für Hygiene," 1899, XXXIII, p. 239.

‡ "Berliner klin. Wochenschrift," 1893 and 1894; "La Riforma Medica," 1892 and 1893; "Centralbl. f. Bakt.," etc., 1890–91.

§ "Zeitschrift für Hygiene," 1893, XIII, p 407.

If the white mouse is used for testing, one unit must protect against 300,000 minimum fatal doses of toxin.

The experiments of Lambert* show that a protective power of 1 : 800,000,000 can be attained.

This method of testing was first improved by Knorr† and finally made exact and practical according to the ideas of Ehrlich, by Marx.‡

The arbitrary standard unit is contained in a serum carefully tested by the old method until its strength was accurately determined. It is dried, pulverized, and sealed in a vacuum tube with phosphoric anhydride, as in the case of the diphtheria standard test serum, and periodically a tube is opened and the contents dissolved, exactly as in the case of the diphtheria test serum. One c.c. of this serum was able to neutralize 40,000,000 times the fatal toxin dose per gram of white mouse, and of it 1 c.c. was said to contain one antitoxic unit.

The test-toxin is prepared from a bouillon culture of the tetanus bacillus, by precipitating with sodium sulphate and desiccating *in vacuo*. The spores of the bacillus collect in immense numbers in the precipitate, and though they do no harm to the animal, not being able to germinate in the body, they interfere with exact results in weighing the powder, so that it is best to redissolve the precipitate, centrifugalize the solution for some time to collect the spores, decant the supernatant fluid, and reprecipitate with the ammonium sulphate. The exact strength of this finally refined dried and desiccated toxin is to be carefully determined each time serums are tested, and its variations noted. Carefully prepared, and protected from external injurious agents, such a serum retains its strength almost without alteration.

When fresh serums are to be tested, two series are to be conducted, one with the standard serum, the other with the unknown. The L+ or test dose of toxin is the least quantity which, after the addition of $\frac{1}{1000}$ antitoxic unit, not being saturated with antitoxin, is able to kill a white mouse in four or five days.

$$\frac{1}{1000}\text{ A.-E. } + x_9\text{ Toxin} = L_0.$$

* "New York Med. Jour.," June 5, 1897.

† "Experimentelle Untersuchungen über die Grenzen der Herlungesmöglichkrit des Tetanus durch Tetanusheilserum," Marburg, 1895.

‡ "Mitteilungen aus der prüfungstechinschen Praxis," Festschrift zum 60 Geburtstage von Robert Koch, Jena, 1903.

In the first series of tests, the same quantity of the test serum is mixed with quantities of the toxin varying from the point of neutralization (L_0) to more than the test dose ($L+$) until the test dose is determined. The second series, by which the strength of the unknown serum is actually determined, is made by mixing with the test dose of toxin varying quantities of the serum according to its supposed value, until the least quantity is determined that will save the white mouse from death from tetanus on the fourth and fifth day.

The actual test may be carried out as follows*:

$\frac{1}{100}$ c.c. of the test toxin contains 40,000 + minimal fatal doses per gram of white mouse. From the serum whose antitoxic value has been proved various dilutions are prepared (1:100; 1:95; 1:80, etc.). 1 c.c. of each of these is placed in an Erlenmeyer flask together with 1 c.c. of the test toxin and 38 c.c. of distilled water. After the mixture has stood one-half hour, each mouse receives 0.4 c.c. under the skin. If the mouse receiving the 1:100 dilution dies in four or five days, and the mouse with the 1:80 dilution remains well and that with 1:90 dilution acquires a slight tetanus, a new dilution 1:85 must be made and tested. If the mouse receiving it remains well, the value of the serum may be calculated thus: $\frac{1 \text{ c.c.}}{100.85} = \frac{1}{1000}$ A. E.

The white mouse is the animal found best adapted to the test, just as the guinea-pig is for diphtheria tests. The mixtures of toxin and antitoxin are allowed to stand for three-quarters of an hour in the incubator before being injected into the mouse, this being made necessary by the fact that the tetanus toxin has far less avidity for antitoxin than diphtheria toxin for diphtheria antitoxin. The injections are made into the skin of the back. At the Frankfort-on-the-Main Institute this method of testing is said to be exact within 5 or 6 per cent.

Numerous cases of the beneficial action of this antitoxin are on record, but, as Welch † has pointed out, the antitoxin of tetanus has proved a disappointment in the treatment of tetanus. Moschcowitz,‡ in his excellent literary review of the subject, has shown that its use has reduced the death-rate from about 80 to 40 per cent., and that it therefore cannot be looked upon as a failure. The result of its experimental injection, in combination with the toxin, into mice, guinea-pigs, rabbits, and other animals is perfectly satisfactory, and affords protection against

* See v. Lingelsheim's paper in Kolle and Wassermann's "Pathogene Mikroorganismen," Bd. IV, Th. 2, p. 989.

† "Bulletin of the Johns Hopkins Hospital," July and August, 1895

‡ "Annals of Surgery," 1900, XXXII, 2, pp. 219, 416, and 567.

almost any multiple of the fatal dose, but the quantity needed, in proportion to the body-weight, to save an animal from the unknown quantity of toxin being manufactured in its body increases so enormously with the day or hour of the disease as to make the dose, which increases millions of times where that of diphtheria antitoxin increases but tenfold, a matter of difficulty and uncertainty. Nocard also called attention to the fact that the existence of tetanus cannot be known until a sufficient toxemia to produce spasms exists, and that therefore it is impossible to attack the disease in its inception or to begin the treatment until too late to effect a cure. At this point it is well to recall Nocard's experiment with the sheep in whose blood so much toxin was already present when symptoms first appeared, that the amputation of their infected tails could not save them.

The explanation of this inability of the antitoxin to effect a cure when administered after development of the symptoms of tetanus is probably found in a ready fixation of the toxin in the bodies of the infected animals. This is well shown by the experiments of Dönitz,* who found that if a mixture of toxin and antitoxin were made before injection into an animal, twelve minimum fatal doses were neutralized by 1 c.c. of a 1 : 2000 dilution of an antitoxin. If, however, the antitoxin was administered four minutes after the toxin, 1 c.c. of a 1 : 600 dilution was required; if eight minutes after, 1 c.c. of a 1 : 200 dilution; if fifteen minutes after, 1 c.c. of a 1 : 100 dilution. He found that similar but slower fixation occurred with diphtheria toxin.

It was found by Roux and Borrel † that doses of tetanus antitoxin absolutely powerless to affect the progress of the disease, when administered in the ordinary manner by subcutaneous injection, readily saved the animal if the antitoxin were injected into the brain substance.

This observation was followed by a ready application to human medicine, and patients with tetanus were trephined and the antitoxin injected beneath the dura and into the cerebral substance. The results attained have not, however, been satisfactory, and the method cannot be looked upon as itself free from danger.

Chauffard and Quénu,‡ who injected the antitoxin into

* Reference 18, in "Jour. of Hygiene," vol. II, No. 2, in Ritchie's article.

† "Ann. de l'Inst. Pasteur," 1898, No. 4.

‡ "La Presse méd.," No. 5, 1898.

the cerebral substance, found that such administration brought about an apparent cure in one case.

Prophylactic Treatment.—While tetanus antitoxin is extremely disappointing in practice for the cure of tetanus, it is most satisfactory for its prevention. "An ounce of prevention is better than a pound of cure," and if the surgeon would administer a prophylactic injection of tetanus antitoxin in every case in which the occurrence of tetanus was at all likely, the disease would rarely develop.

Bacilli Resembling the Tetanus Bacillus.

Tavel * has called attention to a bacillus commonly found in the intestine, sometimes in large numbers in the appendix in cases of appendicitis, and looked upon by one of his colleagues, Fräulein Dr. von Mayer, as the probable common cause of appendicitis. He calls it the "Pseudo-tetanus-bacillus."

The bacillus is slender and measures 0.5 by 5–7 μ, is rather more slender than the tetanus bacillus, and its spores are oval, situated at the end of the rod, and cause a slight bulging rather pointed at the end. The bacillus is provided with not more than a dozen flagella,—usually only four to eight,—thus differing markedly from the tetanus bacillus, which has many. The flagella are easily stained by Löffler's method without the addition of acid or alkali. The organism does not stain so well by Gram's method as the true tetanus bacillus. The bacillus is a pure anaerobe.

The growth in bouillon is rather more rapid than that of the tetanus bacillus. It will not grow in gelatin. The growth in agar-agar is very luxuriant and accompanied by the evolution of gas. Upon obliquely solidified agar-agar the colonies are round, circumscribed, and often encompassed by a narrow, clear zone, which is often notched. The organism grows in serum only in a vacuum. The spores are killed at 80° C.

The organism produced no symptoms in mice, guinea-pigs, and rabbits even when 2–5 c.c. of a culture were subcutaneously introduced.

Sanfelice † and Lubinski ‡ have observed a bacillus in

* "Centralbl. f. Bakt.," etc., March 31, 1898, XXIII, No. 13, p. 538.

† "Zeitschrift für Hygiene," vol. XIV.

‡ "Centralbl. f. Bakt. u. Parasitenk.," XVI, 19.

earth and meat-infusions that is morphologically and culturally like the tetanus bacillus, but differs from it in not possessing any pathogenic powers.

Kruse * has also described a bacillus much like the tetanus micro-organism that grows aerobically. It is not pathogenic.

* Haggis, "Die Mikroorganismen," vol. II, p. 267.

CHAPTER II.

HYDROPHOBIA, LYSSA, OR RABIES.

THERE can be no doubt about the specific infectious nature of rabies, though up to the present time its cause has not been discovered.

Many have labored upon the problems of hydrophobia, but no name is so well known or so justly honored as that of the great pioneer in bacteriology, Pasteur. The profession and laity are alike familiar with his name and work, and although at times the newspapers of our country and certain members of the profession have opposed the method of treatment which he suggested, we cannot but feel that this skepticism and opposition are due to ignorance of the principles upon which Pasteur reasoned.

Hydrophobia, lyssa, or rabies, is a specific infectious toxic disease to which dogs, wolves, skunks, and cats are highly susceptible, and which, through their saliva, can be communicated to men, horses, cows, and other animals. The means of communication is almost invariably a bite, hence the specific organism must be present in the saliva.

The infected animals manifest no symptoms during a varying incubation period in which the wound heals kindly. This period may be of twelve months' duration, but in rare cases may be only a few days. The average duration of the period of incubation is about six weeks.

Toward the close of the incubation period an observable alteration occurs in the wound, which becomes reddened, may suppurate, and is painful. The victim has a sensation of horrible dread, which passes into wild excitement, with paralysis of the pharyngeal muscles and inability to swallow. The wild delirium ends in a final stage of convulsion or palsy. The convulsions are tonic, rarely clonic, and finally cause death by interfering with respiration.

During the convulsive period much difficulty is experienced in swallowing liquids, and it is supposed that the popular term "hydrophobia" arose from the reluctance of

the diseased to take water because of painful spasms caused by the attempt.

This brief description will suffice to illustrate a parallelism existing between hydrophobia and tetanus. In both affections we observe the entrance of infectious material through a wound, which sometimes heals, but often suppurates a little. We see in both affections an incubation period of varying duration, though in hydrophobia it is much longer than in tetanus, and in both there occur convulsions of tonic character, causing death from asphyxia.

It is maintained by some that the stage of excitement argues against the specific nature of the disease, and that it is essentially hysteric; but these subjective symptoms are like the mental condition of tuberculosis, which leads the patient to make a hopeful prognosis of his case, and the mental condition of anthrax, in which it is said that no matter how dangerous his condition the patient is seldom much alarmed about it.

Pasteur * and his co-workers, Chamberland and Roux,† found that in animals that die of rabies the salivary glands, the pancreas, and the nervous system contain the infection, and are more appropriate for experimental purposes than the saliva, which is invariably contaminated with accidental pathogenic bacteria.

The introduction of a fragment of the medulla oblongata of a dog dead of rabies, beneath the dura mater of a rabbit, causes the development of typical rabies in the rabbit in about six days. It is only by such an inoculation that a positive diagnosis of the disease can be made. The operation must be performed with the greatest care in order to avoid septic infection with meningitis. The technic is simple, a small trephine for opening the rabbit's skull being obtainable from the dealers, though in its absence the thin bone of the cranial cavity may be cut with a heavy scalpel. The material to be inoculated should be crushed to a fine pulp in sterile physiologic salt solution, and introduced beneath the dura with a hypodermic syringe. The tissue of the medulla of a rabid rabbit introduced beneath the dura mater of a second rabbit produces a more violent form of the disease in a shorter time, and by frequently repeated implantations Pasteur found that an extremely virulent material could be obtained.

* "Compte-rendu Acad. des Sciences," Paris, 1889, CVIII, p. 1228.

† *Ibid.*, Oct. 26, 1885.

Inasmuch as the toxins of diphtheria and tetanus circulate in the blood, and not infrequently saturate the nervous systems of affected animals, it might be inferred that the material thus producing the rabies is a toxin. This is readily disproved, however, not only by the fact that a toxin would weaken instead of strengthen by transfer from animal to animal, not being vital, but also by the fact that when such an emulsion of the nervous system of an affected animal is filtered through porcelain, its virulence is entirely lost. This seems to prove that the disease depends upon a living, active body—a parasite, and in all probability a bacterium. However, all endeavors to discover, isolate, or cultivate this organism have failed.

Pasteur observed that the virulence of the poison was less in animals that had been dead for some time than in those just killed, and by experiment found that when the nervous system of an infected rabbit was dried in a sterile atmosphere its virulence attenuated in proportion to the length of time it was kept. A method of attenuating the virulence was thus suggested to Pasteur, and the idea of using it as a protective vaccination soon followed. After careful experimentation he found that by inoculating a dog with much attenuated, then with less attenuated, then with moderately strong, and finally with a strong, virus, it developed an immunity that enabled it to resist infection with an amount of virulent material that would certainly kill an unprotected dog.

It is remarkable that this theory, based upon limited accurate biologic knowledge, and upon experience with very few bacteria, should find absolute confirmation as our knowledge of immunity, toxins, and antitoxins progresses. What Pasteur did to produce immunity against rabies is what we now do in producing the anti-serums—*i. e.*, gradually accustom the animal to the poison until its body-cells are able to neutralize or resist it. As in the case of rabies the specific poison cannot be cultivated outside of the body, because the bacilli, micrococci, or whatever they may be, had not yet been discovered, Pasteur introduced the unknown poison-producers, attenuated by drying, and capable of generating only a little poison, accustomed the animal first to them and then to stronger and stronger ones until immunity was established. It was upon this same principle that Behring subsequently began his work upon diphtheria immunization.

The genius of Pasteur did not cease with the production of immunity, but, we are glad to add, extended to the kindred subject of therapy, and gave us a *cure for hydrophobia.*

For the cure of infected cases exactly the same treatment is followed as for the production of immunity. Indeed, the treatment of the disease is simply the production of immunity during the incubation period of the affection, so that the subsequent course is prevented. The patient, to be successfully treated, must come under observation early. The treatment consists of the subcutaneous injection of about 2 grams of an emulsion of a rabbit's spinal cord which had been dried in a sterile bottle over caustic potash for from seven to ten days. This beginning dose is not increased in size, but each day the emulsion injected is made from a rabbit's spinal cord that has not been so long dried, until, when the twenty-fifth day of treatment is reached, the patient receives 2 grams of emulsion of rabbit's spinal cord dried only three days, and is considered immune or cured.

This, in brief, is the theory and practice of Pasteur's system of treating hydrophobia. It is entirely in keeping with the ideas of the present time. When we remember that the first application of the method to human medicine was made October 26, 1885, six years before the time we began to understand the production and use of antitoxins, it becomes one of the most remarkable achievements of medicine.

Frantzius * has studied the bile of animals immunized against rabies, and found it possessed of marked neutralizing effect upon the rabies poison, so that when 0.2 gram of bile and 0.2 gram of comminuted rabid rabbit's medulla are simultaneously introduced beneath the dura of a healthy rabbit, no disease occurs. The bile of healthy oxen, sheep, hogs, etc., was also studied, but found to be without effect. He concludes that the bile is the most powerful rabies antitoxin (?) yet discovered.

The action of the bile in this combination probably corresponds with that discovered by Koch, who found that the bile of cattle suffering from the *Rinderpest,* or South African plague, exerted an immunizing power by which healthy animals could be protected from the disease.

* "Centralbl. f. Bakt. u. Parasitenk.," May 13, 1898, XXII, No. 18.

Högyes, of Budapest,* believes that Pasteur was mistaken in supposing that the drying was of importance in attenuating the virus, and thinks that dilution is the chief factor. He makes an emulsion of rabbit's medulla (1 gram of medulla to 10 c.c. of sterile broth) as a stock solution, to be prepared freshly every day, and uses it for treatment, the first dilution used being 1 : 10,000; then on succeeding days 1 : 8000, 1 : 6000, 1 : 5000, 1 : 2000, 1 : 1000, 1 : 500, 1 : 250, 1 : 200, 1 : 100, and finally the full strength, 1 : 10.

Cabot † prepared a stock solution of 8 parts of rabbit's brain and 80 parts of glycerin and water. The quantity of glycerin added comprised one-fifth of the total bulk. After the emulsion was made it was filtered through sterile cheese-cloth. This emulsion containing the glycerin, if kept in the ice-chest, will be of standard virulence during the entire period of immunization. As the result of his experiments, Cabot found the dilution method attended with danger to the animal immunized, which is not true of the dried-cord method of Pasteur. The latter method is therefore the one to be preferred.

Though the essential cause of rabies has not yet been discovered, its lesions can be found in the medulla oblongata and in the spinal ganglia. These consist in certain cellular aggregations known as the "tubercles of Babes," and in certain degenerative changes in the ganglionic nerve-cells. The regularity with which these changes were observed by Babes led him to regard them as useful for diagnosticating the disease, and Van Gehuchten and Nélis, and Ravenel and McCarthy have confirmed this opinion. Ravenel and McCarthy ‡ think that Babes gives undue prominence to the rabic tubercle, which consists of an aggregation of embryonal cells about the central canal of the cord, about the ganglionic nerve-cells, and about the capillary blood-vessels, but that the lesions of the nerve-cells are pathognomonic if taken in connection with the clinical manifestations of the disease. These ganglion-cell changes consist in degeneration and chromatolysis. There is loss of the prolongation and a progressive modification, and even total disappearance of the nuclei, a dilatation of

* Acad. des Sciences de Buda-Pest, Oct. 17, 1897; "Centralbl. f. Bakt. u. Parasitenk.," 1887, II, 579.

† "Journal of Experimental Medicine," 1899, vol. IV, No. 2.

‡ "Trans. Phila. Pathological Society," N. S., vol. III, 1900, p. 231; and "University Medical Magazine," Jan., 1901.

the pericellular space, and an invasion not only of this space, but also of the nerve-cells by embryonal cells, and at the same time small corpuscles which are hyaline, brownish, and in parts metachromatic. Spiller * does not regard the lesions as pathognomonic of rabies.

If an accurate diagnosis of rabies can be made, in cases where animals thought to be mad have bitten human beings, by a simple histologic examination, much time can be saved in beginning the Pasteur treatment and probably an increased number of cases saved.

Protozoa and Hydrophobia.—The inability of the modern methods to enable us to demonstrate the presence of bacteria in rabies, has led many to conclude that the disease depends upon some animal parasite, and the recent contribution of Negri † is very suggestive.

Negri found that by staining sections of the cerebrum, cerebellum, pons, basal ganglia and sometimes even the spinal ganglia, of human beings or animals dead of rabies, by Mann's methylene-blue and eosin method, it was possible to demonstrate in the interior of the nerve-cells of their protoplasmic process, red colored, rounded bodies measuring 4 to 10 μ, as a rule, though varying from 1 to 25 μ, unlike anything seen before and highly suggestive of parasites. In experimental infections under the dura they were most numerous in the hippocampal convolution. These bodies have now become known as Negri's bodies.

As the parasitic nature of these bodies is suggested only by the circumstances under which they are found and their morphology, it is not demonstrated that they are of etiologic importance.

Anti-rabic Serum.—The serum of animals that have received repeated injections of the crushed nervous tissue of rabid animals is neutralizing or destructive to the rabies virus *in vitro*, and is called anti-rabic. It was first mentioned by Babes and Lepp,‡ who thought it exerted a depressive power upon other animals. Marie § finds that this reaction is specific as simple neurotoxic serum—*i. e.*, serum of an animal given repeated injection of crushed normal nervous tissue—is inert in its action upon the virus. The activity of the anti-rabic serum is also found to be in proportion to the virulence of the virus, and quantity of the virus introduced into the experiment animal.

* Pathological Society of Philadelphia, March, 1901.

† "Soc. Med.-chirurg. di Pavia," 24, III, 1903.

‡ "Ann. de l'Inst. Pasteur," 1889, III.

§ "Compt. rendu Soc. Biol.," t. LVI, June 18, 1904, p. 1030.

CHAPTER III.

DIPHTHERIA.

Bacillus Diphtheriæ (Klebs-Löffler).

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-chromogenic, non-liquefying, aerobic, purely parasitic, pathogenic, toxicogenic bacillus, cultivable upon the ordinary culture media, staining by the ordinary methods and by Gram's method.

In 1883 Klebs * demonstrated the presence of a bacillus in the pseudo-membranes upon the fauces of patients

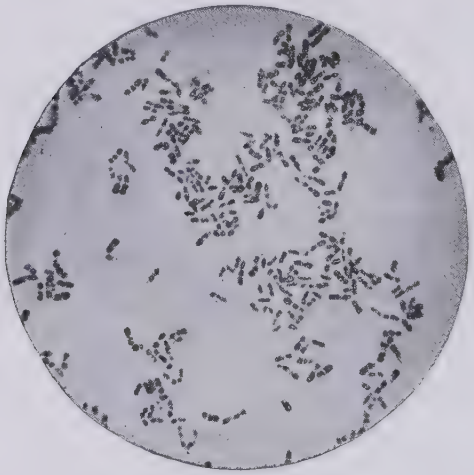


Fig. 122.—Bacillus diphtheriæ, from a culture upon blood-serum. × 1000 (Fränkel and Pfeiffer).

suffering from diphtheria, but it was not until 1884 that Löffler † succeeded in isolating and cultivating it. The organism is now known by both their names, and called the Klebs-Löffler bacillus.

Morphology.—The bacillus is about the length of the tubercle bacillus (1.5–3.5 μ), but about twice its diameter (0.4–1.0 μ), has a slight curve similar to that which characterizes the tubercle bacillus, and has rounded and usually clubbed ends (Fig. 122). It does not form chains, though two, three, and rarely four individuals may be found con-

* "Verhandlungen des Congresses für innere Med.," 1883.
† "Mittheilungen aus dem kaiserlichen Gesundheitsamt," 2.

joined; usually the individuals are separate from one another. The bacillus is peculiar in its pleomorphism, for among the well-formed individuals which abound in fresh cultures a large number of peculiar organisms are to be found, much larger than normal, some with one end enlarged and club shaped, some greatly elongated, with both ends similarly and irregularly expanded. These bizarre

Fig. 123.—Wesbrook's types of Bacillus diphtheriæ: *a*, *c*, *d*, Granular types; a^1, c^1, d^1, barred types; a^2, c^2, d^2, solid types. × 1500.

forms probably represent an involution form of the organism, for, while present in perfectly fresh cultures, they are much more abundant in old cultures where scarcely a single well-formed bacillus can be found. Distinct polar granules can be defined at the ends of the bacilli. Occasional branched forms are observed, and the diphtheria bacillus probably belongs to the higher bacteria, though Abbott and Gildersleeve* do not regard branching as a phase of the normal development of the organism, do not find it common upon the standard culture media, and so do not think that it is properly classified elsewhere than among the bacilli.

No flagella have been demonstrated upon the bacillus, and it is non-motile. It is almost purely aerobic.

The involution of the diphtheria bacillus seems to occur in proportion to the rapidity of its growth. Upon Löffler's serum mixture, which seems best adapted for its cultivation,

*"Centralbl. f. Bakt.," etc., Dec. 18, 1903, Bd. xxxv, No. 3.

the involution of the organism takes place with great rapidity, so that large clubbed organisms and large organisms with polar granules are very common. On the other hand, upon agar and glycerin agar-agar, where the organism grows very slowly, it usually appears in the form of short spindle and lancet shapes. So different are these forms that a beginner would certainly fail to recognize them as identical. The small short forms also stain much more uniformly than the large club-shaped bacilli.

Wesbrook * has established certain morphologic types of the bacillus (see illustration), and from the appearances presented draws conclusions regarding their virulence, which are confirmed by Gorham,† but disputed by Denny.‡ The rapidly growing bacilli with clubbed ends and polar granules are supposed to be virulent forms; the slowly growing, uniformly staining forms, non-virulent bacilli. Park and Denny believe that the uniformly staining bacillus, when it develops in blood-serum cultures, is the pseudo-diphtheria bacillus, an entirely different organism.

Staining.—The bacillus can readily be stained with aqueous solutions of the anilin colors, but more beautifully and characteristically with Löffler's alkaline methylene-blue:

Saturated alcoholic solution of methylene-blue	30
1 : 10,000 aqueous solution of caustic potash	100

or in an aqueous solution of dahlia, as recommended by Roux.

The Neisser method of staining the diphtheria bacillus, which met with a very cordial reception, is as follows:

The prepared cover-glass is immersed for from two to three seconds in

Alcohol (96 per cent.)	20 parts
Methylene-blue	1 part
Distilled water	950 parts
Acetic acid (glacial)	50 "

Then for three to five seconds in

Bismarck brown	1 part
Boiling distilled water	500 parts

* "Trans. Assoc. Amer. Phys.," 1900, and "Trans. of the Amer. Public Health Assoc.," 1900.

† "Journal of Medical Research," N. S., vol. 1, p. 201, 1901.

‡ American Public Health Association (New Orleans) meeting, 1902.

The true diphtheria bacilli appear brown, with a dark blue body at one or both ends; the pseudo-diphtheria bacilli usually exhibit no polar bodies.

Park * in his large experience found that neither the Neisser nor the Roux stain gave any more information concerning the virulence of the bacilli than the Löffler alkaline methylene-blue.

When cover-glass preparations are stained with these solutions, the bizarre forms already mentioned are particularly obvious, and the contrast between the polar granules, which color intensely, and the cytoplasm of the bacillus, which tinges slightly, is marked. Through good lenses the organisms are always distinct bacilli, notwithstanding the fact that the ends stain more deeply than the centers, and it is only through poor lenses that the organisms can be mistaken for diplococci.

The bacilli stain well by Gram's method, which is excellent for their definition in sections of tissue, though Welch and Abbott found that Weigert's fibrin method and picrocarmin gave the most beautiful results.

Cultivation.—The diphtheria bacillus grows readily upon all the ordinary media, and is very easy to obtain in pure culture, plates not being necessary. Material from the infected throat can be taken with a swab or platinum loop and spread upon the surface of several successive tubes of Löffler's blood-serum media. Upon the first a confluent growth of the bacillus usually occurs; but upon the third, scattered colonies suitable for transplantation can usually be found.

Löffler has shown that the addition of a small amount of glucose to the culture medium increases the rapidity of growth, and suggests a special medium which bears his name—Löffler's blood-serum mixture:

Blood-serum	3
Ordinary bouillon + 1 per cent. of glucose	1

This mixture is filled into tubes, coagulated, and sterilized like blood-serum, and is one of the best known media to be used in connection with the study of diphtheria.

The studies of Michel † have shown that the development

* "Bacteriology in Medicine and Surgery," 1900.

† "Centralbl. f. Bakt. u. Parasitenk.," Sept. 24, 1897, Bd. XXII, Nos. 10 and 11.

of the culture is much more luxuriant and rapid when horses' serum instead of beef or calves' serum is used. Horse's blood can easily be secured by the introduction of a trocar into the jugular vein; 5 liters of it can be withdrawn without causing the animal inconvenience or symptoms of weakness.

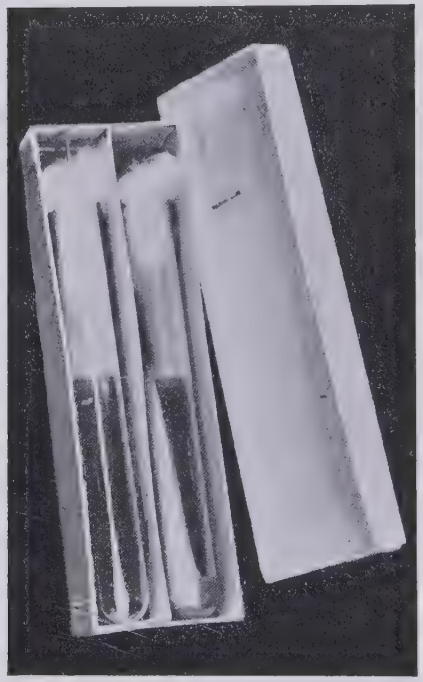


Fig. 124.—The Providence Health Department outfit for diphtheria diagnosis, consisting of a pasteboard box containing a swab-tube and a serum-tube, both with etched surface on which to write the name and address of patients, etc.

The impossibility of making an accurate diagnosis of diphtheria without a bacteriologic examination has caused many private physicians and many medical societies and boards of health to equip laboratories where bacteriologic examinations can be made. The method requires some apparatus, though a competent bacteriologist can often make shift with a bake-oven, a wash-boiler, and other household furniture, instead of the regular sterilizers and incubators, which are expensive.

Bacteriologic Diagnosis.—When it is desired to make a bacteriologic diagnosis in suspected diphtheria, or to secure the bacillus in pure culture, a sterile platinum wire having a small loop at the end, or a swab made by wrapping a little absorbent cotton about the end of a piece of wire and carefully sterilizing it in a test-tube, is introduced into the throat and touched to the false membrane, after which it is carefully smeared over the surface of at least three of the blood-serum mixture tubes, without either

again touching the throat or being sterilized. The tubes thus inoculated are stood away in an incubating oven at the temperature of 37° C. for twelve hours, then examined. If the diphtheria bacillus be present, a smeary, yellowish-white layer will be present upon the first tube, a similar layer with outlying colonies on the second tube, while the third tube will show rather large, isolated, whitish or slightly yellowish, smooth colonies. The colonies may be china-white in appearance. These colonies, *if found by microscopic examination to be made up of diphtheria bacilli*, will confirm the diagnosis of diphtheria, and will at the same time give pure cultures of the bacillus when transplanted. There are very few other bacilli that grow so rapidly upon Löffler's mixture, and scarcely any other is found in the throat.

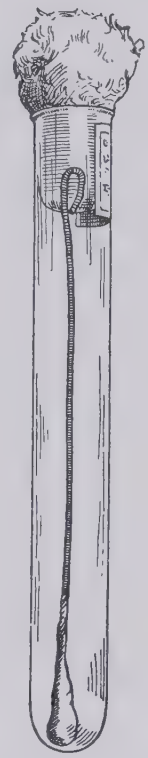


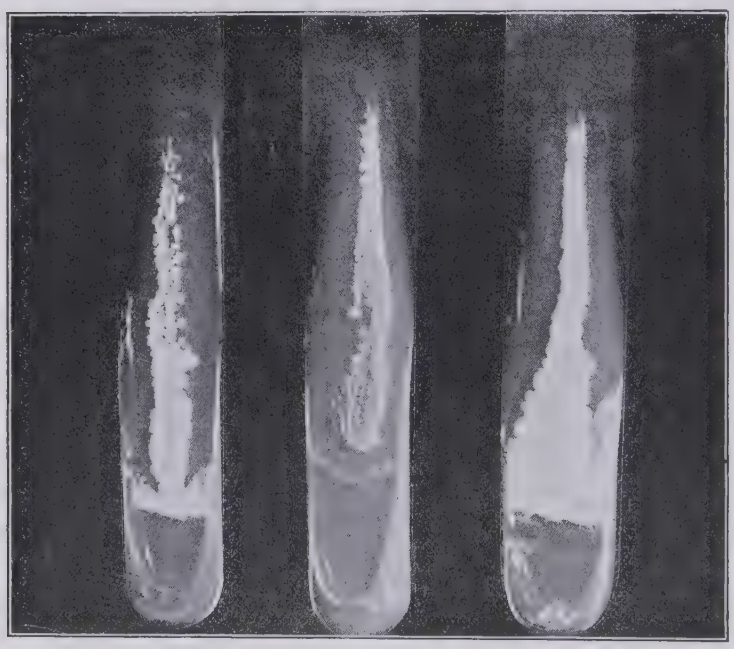
Fig. 125 — Sterilized test-tube and swab for collecting pus and fluids for bacteriologic examination (Warren).

When no tubes of the blood-serum mixture are at hand, the swab can be returned to its tube after having been wiped over the throat of the patient, and can be shipped to the nearest laboratory.

When an early diagnosis is required, Ohlmacher recommends that the microscopic examination of the still invisible growth be made in five hours. A platinum loop is rubbed over the inoculated surface; the small amount of material thus secured is mixed with distilled water, spread on a cover-glass, dried, fixed, stained with methylene-blue, and examined. An abundance of the organisms are usually found and valuable time is saved preparatory to the use of the antitoxin.

The presence of diphtheria bacilli in material taken from the throat does not necessarily mean that the person has

diphtheria. Virulent bacilli can occasionally be discovered in the throats of healthy persons who have knowingly or unknowingly come in contact with the disease, but whose vital resistance is such that the bacilli grow scantily without producing disease of the throat. The bacteriologic examination is, therefore, only an adjunct to the clinical diagnosis, and must never be taken as positive in itself.



a *b* *c*

Fig. 126.—Diphtheria bacilli (from photographs taken by Prof. E. K. Dunham, Carnegie Laboratory, New York): *a*, Pseudobacillus; *b*, true bacillus; *c*, pseudobacillus.

Gelatin.—Gelatin is not an appropriate medium for the cultivation of the bacillus. Upon the surface of gelatin plates the colonies attain but a small size and appear to the naked eye as whitish points with smooth contents and regular, though sometimes indented, borders. Under the microscope they appear granular and yellowish-brown, with

irregular borders (Fig. 127). The growth in gelatin puncture is characterized by the occurrence of small spheric colonies along the line of inoculation. The gelatin is not liquefied.

Agar-agar.—Upon agar-agar and glycerin agar-agar the colonies are slower to develop, larger, more translucent, without the yellowish-white or china-white color of the blood-serum cultures, and are more or less distinctly divided into a small elevated center and a flat surrounding zone with indented edges, and a radiated appearance. When transplantations are made from blood-serum to agar-agar, the resulting growth is usually meager, but the oftener the organism is transplanted to fresh agar-agar, the more luxuriant its growth becomes.

Bouillon.—When planted in bouillon a distinct, whitish, granular pellicle forms upon the surface of the medium, especially when the culture is freely exposed to the air or made by the method of Fernbach with a passing current of air. This pellicle appears quite uniform when the flask is

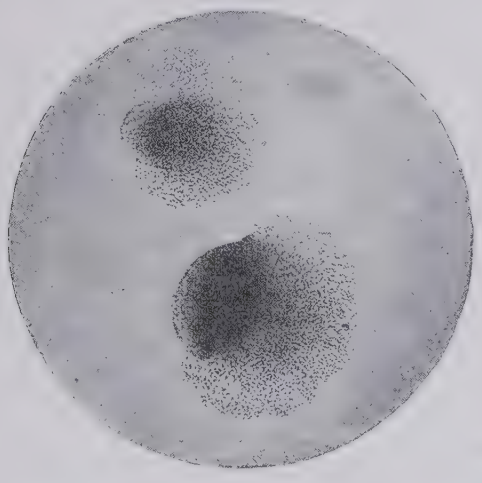


Fig. 127.—Bacillus diphtheriæ; colony twenty-four hours old, upon agar-agar. × 100 (Fränkel and Pfeiffer).

undisturbed, but is so brittle that it at once falls to pieces if the flask be moved, the minute fragments slowly sedimenting and forming a miniature snow-storm in the flask or tube. The organism at times also causes a diffuse cloudiness of the medium, but, not being motile, soon settles to the bottom

in the form of a flocculent precipitate which has a tendency to cling to the sides of the glass, and leave the bouillon clear.

Spronck * found that the characteristics of the growth of the diphtheria bacillus in bouillon, as well as the amount of toxin produced, vary according to the amount of glucose in the bouillon. He divides the cultures into three types:

Type A. The reaction of the bouillon becomes and remains acid, the acidity increasing. The bacilli accumulate at the bottom of the clear liquid. The toxin-production is meager.

Type B. There is no change from alkalinity to acidity, but the original alkalinity of the bouillon steadily increases. The culture is very rich, the bottom of the flask shows a considerable sediment, the liquid is cloudy, and a delicate growth occupies the surface. The toxicity is very great.

Type C. In a few days the reaction of the culture becomes acid, and then later on changes to alkaline. During the acid period the liquid is clear, with a white surface growth. When the alkalinity returns, the bouillon clouds and the surface growth increases in thickness. Sediment accumulates at the bottom of the flask. The toxicity of the culture is much less than in Type B.

Spronck regards the varying reactions as depending upon the fermentation of the glucose, and asserts that the most luxuriant and toxic cultures are those grown in bouillon in which no glucose is present. To exclude as much of the undesirable sugar as possible, he makes the bouillon from the stalest meat obtainable, preferring to secure it when just about to putrefy. Of the meats experimented with, beef was found to be the best.

Zinno† found that digested brain added to the culture bouillon greatly facilitated the growth of diphtheria and tetanus bacilli and increased the toxin-production.

Blood-serum.—The bacillus grows similarly upon blood-serum and Löffler's mixture.

Potato.—Upon potato it develops only when the reaction is alkaline. The potato growth is not characteristic.

Milk.—Milk is an excellent medium for the cultivation of Bacillus diphtheriæ, and is possibly at times a medium of infection. Litmus milk is useful for detecting the changes of reaction brought about by the alkalinity, which at first favors the development of the bacillus, being soon replaced by acidity. When the culture becomes old, the reaction again becomes strongly alkaline. This variation in reaction seems to depend entirely on the transformation of the sugars.

* "Ann. de l'Inst. Pasteur," Oct. 25, 1895, vol. IX, No. 10, p. 758.
† "Centralbl. f. Bakt.," Jan. 4, 1902, XXXI, No. 2, p. 42.

Vital Resistance.—The diphtheria bacillus does not form spores. It possesses very little vital resistance and is delicate in its thermic sensitivity. Löffler found that it could not endure a temperature of 60° C., and Abbott has shown that a temperature of 58° C. is fatal to it in ten minutes. The organism can sometimes be kept alive for several weeks after being dried upon shreds of silk or when surrounded by dried diphtheria membrane.

Metabolic Products.—The earliest researches upon the nature of the poisonous products of the diphtheria bacillus seem to have been made in 1887 by Löffler,* who came to the conclusion that they belonged to the enzymes. The credit of removing the bacteria from the culture by filtration through porcelain and the demonstration of the soluble poison in the filtrate belongs to Roux and Yersin.† Toxic bouillon prepared in this manner was found to cause serous effusions into the pleural cavities, acute inflammation of the kidneys, fatty degeneration of the liver, and edema of the tissue into which the injection was made. In some cases palsy subsequently made its appearance, usually in the hind quarters. The effect of the poison was slow and death took place days or weeks after injection, sometimes being preceded by marked emaciation. Temperatures of 58° C. lessened the activity of the toxin and temperatures of 100° C. destroyed it. It was precipitated by absolute alcohol and mechanically carried down by calcium chlorid. Brieger and Fränkel ‡ confirmed the work of Roux and Yersin, and concluded that the poison was a toxalbumin. Tangl § was able to extract the toxin from a fragment of diphtheria pseudo-membrane macerated in water.

The nature of the diphtheria toxin has been studied by Ehrlich ‖ and found to be extremely complex. As it exists in cultures it is composed of equal parts of toxin and toxoid. Of these, the former is poisonous, the latter harmless for animals—or at least not fatal to them. The toxoids have equal or greater affinity for combining with antitoxin than the toxin and cause confusion in testing the unit value or strength of the antitoxin. In old or heated toxin all

* "Centralbl. f. Bakt.," etc., 1887, II, p. 105.
† "Ann. de l'Inst. Pasteur," 1888–1889.
‡ "Berliner klin. Wochenschrift," 1890, 11–12.
§ "Centralbl. f. Bakt.," etc., Bd. XI, p. 379.
‖ "Klinisches Jahrbuch," 1897.

of the toxin molecules become changed into toxons or toxoids and the poisonous quality is lost though the power of combining with antitoxin remains.

The toxin is intensely poisonous, and a filtered bouillon containing it may be fatal to a 300-gram guinea-pig in doses of only 0.0005 c.c. It is thought not to be an albuminous substance, as it can be elaborated by the bacilli when grown in non-albuminous urine, or, as suggested by Uschinsky, in non-albuminous solutions whose principal ingredient is asparagin. The toxic value of the cultures is greatest in the second week.

This soluble toxin so well known in bouillon cultures is probably only one of the poisonous substances produced by the bacillus. An intracellular, insoluble toxic product seems to have been discovered by Rist,* who found it in the bodies of dried bacilli, and observed that it was not neutralized by the antitoxin.

Palmirski and Orlowski† assert that the bacillus produces indol, but only after the third week. Smith,‡ however, found that when the diphtheria bacillus grew in dextrose-free bouillon no indol was produced.

The acidity of the culture media depends upon the formation of lactic acid.

Pathogenesis.—Diphtheria in man is characterized by a pseudo-membranous inflammation of the mucous membranes, particularly of the fauces, though it may occur upon other parts of the body and is not infrequent in the nose, in the mouth, upon the genital organs, or upon wounds. Williams§ has reported a case of diphtheria of the vulva, and Nisot and Bumm have reported cases of puerperal diphtheria from which the bacilli were cultivated. It is in nearly all cases a purely local infection, depending upon the presence and development of the bacilli upon the diseased mucous membrane, but is accompanied by a serious intoxication resulting from the absorption from the local lesions of a poisonous metabolic product of the bacilli. The bacilli

*"Soc. de Biol. Paris," 1903, No. 25.

†"Centralbl. f. Bakt. u. Parasitenk.," March, 1895.

‡"Jour. of Experimental Medicine," Sept., 1897, vol. II, No. 5, p. 546.

§"Amer. Jour. of Obstet. and Dis. of Women and Children," Aug., 1898.

are found only in the membranous exudation, and are most plentiful in its older portions.

The entrance of the diphtheria bacillus into the internal organs can scarcely be regarded as a frequent occurrence, though in severe cases it is not rare.

The disease pursues a course of variable length, in favorable cases the patient recovering gradually, the pseudomembrane first disappearing, leaving an inflamed mucous membrane behind it, upon which virulent diphtheria bacilli persist, always for weeks and sometimes for months. Smith* describes the bacteriologic condition of the throat in diphtheria as follows: "The microscope informs us that during the earliest local manifestations the usual scant miscellaneous bacterial flora of the mucosa is quite suddenly replaced by a rich vegetation of the easily distinguishable diphtheria bacillus. Frequently no other bacteria are found in the culture-tube. This vegetation continues for a few days, then gradually gives way to another flora of cocci and bacilli, and finally the normal condition is reëstablished."

Diphtheria bacilli were first found in the heart's blood, liver, spleen, and kidney, by Frosch.† Kolisko and Paltauf ‡ had already found them in the spleen, and other observers in various lesions of the deeper tissues and occasionally in the organs. In the blood and organs it is commonly associated with Streptococcus pyogenes and sometimes with other bacteria. While present in nearly all of the inflammatory sequelæ of diphtheria, the Klebs-Löffler bacillus probably has very little influence in producing them, the conditions being almost invariably associated with the pyogenic cocci, either the streptococci or staphylococci.

Howard§ studied a case of ulcerative endocarditis caused by the diphtheria bacillus, and Pearce‖ has observed it in 1 case of malignant endocarditis, 19 out of 24 cases of broncho-pneumonia, 1 case of empyema, 16 cases of middle-ear disease, 8 cases of inflammation of the antrum of Highmore, 1 case of inflammation of the sphenoidal sinuses, 1 case of thrombosis of the lateral sinuses, 2 cases of abscesses of the cervical glands, and in esophagitis, gastritis, vulvo-

* "Boston Med. and Surg. Jour.," 1898, I, p. 157.
† "Zeitschrift für Hygiene," etc., 1893, XIII, Heft 1.
‡ "Wiener klin. Wochenschrift," 1889.
§ "Amer. Jour. Med. Sci.," Dec., 1894.
‖ "Jour. Boston Soc. of Med. Sci.," March, 1898.

vaginitis, dermatitis, and conjunctivitis following or associated with diphtheria.

In animals artificially inoculated with the diphtheria bacillus the resulting lesions resemble those seen in the human subject, in that they consist of a local infection with a general toxemia.

Human beings, horses, rabbits, guinea-pigs, mice, kittens, and young pups are susceptible; rats are immune. When half a cubic centimeter of a twenty-four-hour-old bouillon culture is injected beneath the skin of a susceptible animal, the bacilli multiply at the point of inoculation, producing a fibrinous inflammation with edema. The animal dies about the third day. When examined post-mortem the liver is found enlarged and sometimes shows minute whitish points, which upon microscopic examination prove to be necrotic areas in which the cells are completely degenerated, and the chromatin of their nuclei scattered about in granular form. Similar necrotic foci, to which attention was first called by Oertel, are present in nearly all the organs in cases of death from diphtheria intoxication. No bacilli are present in these lesions. Welch and Flexner * have shown these foci to be common to numerous intoxications, and not peculiar to diphtheria.

The lymphatic glands are usually enlarged, and the adrenals enlarged and hemorrhagic. The kidneys show parenchymatous degeneration. There is no inflammation of the fauces.

Roux and Yersin found that when the bacilli were introduced into the trachea of animals opened by operation, a typical pseudo-membrane was formed, and that diphtheritic palsy sometimes followed.

Associated Bacteria.—Streptococcus pyogenes and Staphylococci pyogenes aureus and albus are, in many cases, found in association with the diphtheria bacillus, especially when severe lesions of the throat exist.

In a series of 234 cases carefully and statistically studied by Blasi and Russo-Travali,† it was found that in 26 cases of pseudo-membranous angina due to streptococci, staphylococci, colon bacilli, and pneumococci, 2 patients died, the mortality being 3.84 per cent. In 102 cases of pure diphtheria, 28 died, a mortality of 27.45 per cent. Seventy-

* "Bull. of the Johns Hopkins Hospital," Aug., 1901.
† "Ann. de l'Inst. Pasteur," 1896, p. 387.

six cases showed diphtheria bacilli and staphylococci; of these, 25, or 32.89 per cent., died. Twenty cases showed the diphtheria bacilli and Streptococcus pyogenes, with 6 deaths—30 per cent. In 7 cases, of which 3, or 43 per cent., were fatal, the diphtheria bacillus was in combination with streptococci and pneumococci. The most dangerous forms met were 3 cases, all fatal, in which the diphtheria bacillus was found in combination with Bacillus coli.

In 157 cases of diphtheria and scarlatina studied at the Boston City Hospital by Pearce,* there were 94 cases of diphtheria, 46 cases of complicated diphtheria (29 with scarlet fever, 11 with measles, and 5 with measles and scarlet fever), and 17 cases of scarlet fever (in 3 of which measles was also present).

Of the 94 cases of uncomplicated diphtheria, the Klebs-Löffler bacilli were present in the *heart's blood* in 4, twice alone and twice with streptococci. In 9 cases the streptococcus occurred alone; in 1 case the pneumococcus occurred alone. In the *liver* the bacillus was found in 24 cases, alone in 12 and together with the streptococcus in 12; the streptococcus occurred in 27 cases, alone in 14, with the Klebs-Löffler bacillus in 12, and with Staphylococcus pyogenes aureus in 1. Staphylococcus pyogenes aureus occurred in 4 cases, alone in 3 and associated with the streptococcus in 1. The pneumococcus occurred alone in 1 case.

In the *spleen* the Klebs-Löffler bacillus occurred eighteen times, fifteen times alone and three times associated with the streptococcus. The streptococcus occurred in 24 cases, alone in 21, associated with the Klebs-Löffler bacillus twice, and with Staphylococcus pyogenes aureus once. Staphylococcus pyogenes occurred twice, once alone and once with the streptococcus. The pneumococcus occurred twice alone.

In the *kidney* the Klebs-Löffler bacillus occurred in 23 cases, in 15 alone, in 5 associated with the streptococcus, and in 2 with Staphylococcus pyogenes aureus. The streptococcus occurred in 26 cases, in 19 of which it was the only organism present. Staphylococcus pyogenes aureus occurred in 8 cases, in 4 of which it was in pure culture. The pneumococcus occurred four times, three times in pure culture and once with the Klebs-Löffler bacillus.

In the 46 cases of complicated diphtheria, the *heart's*

* "Jour. Boston Soc. of Med. Sci.," March, 1898.

blood showed pure cultures of the streptococcus nine times and the streptococcus associated with the Klebs-Löffler bacillus once. The diphtheria bacillus occurred alone once.

In the *liver*, in 10 cases streptococcus occurred alone, in 7 cases associated with the Klebs-Löffler bacillus, and in 3 cases with Staphylococcus pyogenes aureus. The diphtheria bacillus occurred in pure culture in 5 cases.

The *spleen* contained streptococci only thirteen times and mixed with the diphtheria bacillus twice. The diphtheria bacillus was found in pure culture in 5 cases.

The *kidney* contained pure cultures of streptococci in 10 cases, streptococci associated with diphtheria bacilli five times, and with Staphylococcus pyogenes aureus three times. The diphtheria bacillus occurred alone in 7 cases. Staphylococcus pyogenes aureus and the pneumococcus each alone once and both together once.

"The clinical significance of this general infection with the Klebs-Löffler bacillus is not apparent. It occurred generally, but not always, in the gravest cases, or those known as 'septic' cases. It is probable that it may be due to a diminished resistance to the tissue-cells, or of the germicidal power of the blood. In this series of fatal cases the number of infections with the streptococcus and with the Klebs-Löffler bacillus was about even, though slightly in favor of the streptococcus."

The mixed infections add to the clinical diphtheria the pathogenic effects of the associated bacteria. The diphtheria bacillus probably begins the process, growing upon the mucous membrane, devitalizing it by its toxin, and producing coagulation-necrosis. Whatever pyogenic germs happen to be present are thus afforded an opportunity to enter the tissues and add suppuration, gangrene, and remote metastatic lesions to the already existing ulceration.

Diphtheritic inflammations of the throat are not always accompanied by the formation of the pseudo-membrane, but in some cases a rapid inflammatory edema in the larynx, without a fibrinous surface coating, may cause fatal suffocation, only a bacteriologic examination revealing the true nature of the disease.

Lesions.—The pseudo-membrane characterizing diphtheria consists of a combined necrosis of the tissues acted upon by the toxin and coagulation of an inflammatory exudate. When examined histologically it is found that

the surface of the mucous membrane is chiefly affected. The superficial layers of cells being embedded in coagulated exudate—fibrin—and show a peculiar hyaline degeneration. Sometimes the membrane seems to consist exclusively of hyaline cells; sometimes the fibrin formation is secondary to or subsequent to the hyaline degeneration. Leukocytes caught in the fibrin also become hyaline. From the superficial layer the process may descend to the deepest layers, all of the cells being included in the coagulated fibrin and showing more or less hyaline degeneration. The walls of the neighboring capillaries also become hyaline, and the necrotic mass forms the diphtheritic membrane. The laminated appearance of the membrane probably depends upon the varying depths affected at different periods, or upon differences in the process by which it has been formed. The pseudo-membrane is continuous with the subjacent tissues by a fibrinous reticulum, and is in consequence removed with difficulty, leaving an abraded surface. When the membrane is divulsed during the course of the disease, it immediately forms anew by the coagulation of the inflammatory exudate.

The coagulation-necrosis seems to depend upon the local effect of the toxin. Morax and Elmassian * found that when strong diphtheria toxin is applied to the conjunctiva of rabbits every three minutes for eight or ten hours, typical diphtheritic changes are produced.

Flexner† has made a study of the minute lesions caused by bacterial toxins and especially of the diphtheria toxin, and Councilman, Mallory, and Pearce,‡ of both gross and minute lesions that the thorough student should read.

Specificity.—Herman Biggs,§ in an interesting discussion of the occurrence of the diphtheria bacillus and its relation to diphtheria, comes to the following conclusions:

1. "When the diphtheria bacillus is found in healthy throats, investigation almost always shows that the individuals have been in contact with cases of diphtheria. The presence of the bacillus in the throat, without any

* "Ann. de l'Inst. Pasteur," 1898, p. 210.

† "Johns Hopkins Hospital Reports," VI, 259.

‡ "Diphtheria: A Study of the Bacteriology and Pathology of Two Hundred and Twenty Fatal Cases," 1901.

§ "Amer. Jour. Med. Sci.," Oct., 1896, vol. XXII, No. 4, p. 411.

lesion, does not, of course, indicate the existence of the disease."

2. "The simple anginas in which virulent diphtheria bacilli are found are to be regarded from a sanitary standpoint in exactly the same way as the cases of true diphtheria."

3. "Cases of diphtheria present the ordinary clinical features of diphtheria, and show the Klebs-Löffler bacilli."

4. "Cases of angina associated with the production of membrane in which no diphtheria bacilli are found might be regarded from a clinical standpoint as diphtheria, but bacteriological examination shows that some other organism than the Klebs-Löffler bacillus is the cause of the process."

Any skepticism of the specificity of the diphtheria bacillus on my own part was dispelled by a somewhat unique experience. Without having been previously exposed to diphtheria while experimenting in the laboratory I accidentally drew a living virulent culture of the diphtheria bacillus through a pipet into my mouth. Through carelessness no precautions were taken to prevent serious consequences, and two days later my throat was filled with typical pseudomembrane which private and Health Board bacteriologic examinations showed to contain pure cultures of the Klebs-Löffler bacilli.

Some have been led to doubt the specificity of the diphtheria bacillus because of the existence of what is called the *pseudo-diphtheria bacillus*. My conviction has always been that this organism is but an attenuated or non-virulent diphtheria bacillus, but it is commonly believed that the two organisms are different, and until some one succeeds in transforming one into the other, the matter must remain an opinion. Bomstein* investigated the question and found that though it was possible to modify the activity of virulent bacilli, and bring back the virulence of non-virulent diphtheria bacilli, it was impossible to make the pseudo-diphtheria bacillus virulent. Denny † also found that the morphology of the two organisms was continually different when they were grown upon the same medium for the same length of time, and that the short pseudo-diphtheria bacillus never showed any tendency to develop into the large clubbed forms

* "Archiv Russes de Path.," etc., Aug. 31, 1902.
† American Public Health Association, 1902.

characteristic of the true diphtheria organism. The chief points of difference between the bacilli are that the pseudo-diphtheria bacillus, when grown upon blood-serum, is short and stains uniformly; that cultures grown in bouillon develop more rapidly at a temperature of from 20°–22° C. than those of the true bacillus; and that the pseudo-bacillus is not pathogenic for animals. These distinctions are, however, exactly what would be expected of an organism whose virulence and vegetative powers had been altered, by persistent manipulation or by unfavorable environment.

Contagion.—The diphtheria bacilli, being always present in the throats of patients suffering from diphtheria, constitute the element of contagion, and by being accidentally discharged from the nose and mouth during coughing, sneezing, vomiting, etc., endanger whoever comes in contact with the patient.

The results obtained by Biggs, Park, and Beebe in New York are of great interest. Bacteriologic examinations conducted in connection with the Health Department of New York city show that virulent diphtheria bacilli may be found in the throats of convalescents from diphtheria, as long as five weeks after the discharge of the membrane and the commencement of recovery, and that they exist not only in the throats of the patients themselves, but also in those of their caretakers, who, while not themselves infected, may be the means of conveying the disease germs from the sick-room to the outer world. Still more extraordinary are the observations of Hewlett and Nolen,* that the bacilli remained in the throats of patients seven, nine, and in one case *twenty-three weeks* after convalescence. The hygienic importance of this observation must be apparent to all readers, and serves as further evidence why thorough isolation should be practised in connection with the disease.

Neumann † found that virulent diphtheria bacilli may occur in the nose with the production of what seems to be a simple rhinitis as well as a pseudo-membranous rhinitis. Such cases, not being segregated, may easily serve to spread the contagion of the disease.

Wesbrook, and Wilson and McDaniel ‡ have found it con-

* "Brit. Med. Jour.," Feb. 1, 1896.

† "Centralbl. f. Bakt. u. Parasitenk.," Jan. 24, 1902, Bd. xxxi, No. 2, p. 41.

‡ "Trans. Assoc. Amer. Phys.," 1900.

venient to describe three chief types of the diphtheria bacillus as it occurs in twenty-four-hour-old cultures on Löffler's blood-serum, sent to the laboratory for diagnosis. The classification places all types in three general groups: (*a*) granular, (*b*) barred, and (*c*) solid or evenly staining forms. Each group is subdivided into types based on the shape and size of the bacilli. A study of variations in the sequence of types in series of cultures derived from clinical cases of diphtheria shows that (*a*) granular types are usually the most predominant forms at the outset of the disease; (*b*) the granular types usually give place wholly or in part to barred and solid types shortly before the disappearance of diphtheria-like organisms; (*c*) solid types, by many observers called "pseudo-diphtheria bacilli," may cause severe clinical diphtheria. Solid types may sometimes be replaced by granular types when convalescence is established and just before the throat is cleared of diphtheria-like bacilli.

From these data the writers conclude that it is not safe to base an opinion regarding the maintenance of quarantine upon the bacterioscopic findings independently of the clinical history of the case.

The occurrence of true diphtheria bacilli in the throats of healthy persons has been a stumbling-block to many practitioners uninformed upon bacteriologic subjects, who fail to account for its presence and also fail to realize how rare its appearance under such circumstances really is.

Park * found virulent diphtheria bacilli in about 1 per cent. of the healthy throats examined in New York city, but diphtheria was prevalent in the city at the time, and no doubt most of the persons in whose throats they existed had been in contact with cases of diphtheria. He very properly concludes that the members of a household in which a case of diphtheria exists, though they have not the disease, should be regarded as possible sources of danger, until cultures made from their throats show that the bacilli have disappeared.

In connection with the contagiousness of diphtheria the recent experiments of Reyes are interesting. He has

* "Report on Bacteriological Investigations and Diagnosis of Diphtheria, from May 4, 1893, to May 4, 1894," "Scientific Bulletin No. 1," Health Department, city of New York.

demonstrated that in absolutely dry air diphtheria bacilli die in a few hours. Under ordinary conditions their vitality, when dried on paper, silk, etc., continues for but a few days, though sometimes they can live for several weeks. In sand exposed to a dry atmosphere the bacilli die in five days in the light; in sixteen to eighteen days in the dark. When the sand is exposed to a moist atmosphere, the duration of their vitality is doubled. In fine earth they remained alive seventy-five to one hundred and five days in dry air, and one hundred and twenty days in moist air.

Diphtheria Antitoxin.—Behring * discovered that the blood of animals rendered immune against diphtheria by inoculation, first with attenuated and then with virulent organisms, contained a neutralizing substance (*Anti-körper*) capable of annulling the effects of the bacilli or the toxin when simultaneously or subsequently inoculated into susceptible animals. This substance, held in solution in the blood-serum of the immunized animals, is the *diphtheria antitoxin*.

The antitoxin is commercially manufactured at present by immunizing horses against increasing quantities of diphtheria toxin until the proper degree of immunity has been attained, then withdrawing the antitoxic blood. The details are as follows:

I. The Preparation of the Toxin.—The toxic metabolic products of the Bacillus diphtheriæ are for the most part freely soluble, and are therefore best prepared in cultures grown in fluid media. The medium best adapted to the purpose is that recommended by Theobald Smith.†

To make it, the usual meat infusion receives the addition of a culture of Bacillus coli, and is stood in a warm place overnight. The colon bacilli ferment and remove the muscle and other sugars. The infusion is then made into bouillon, titrated so that the reaction equals + 1.1 when tested with phenolphthalein. It then receives an addition of 0.2 per cent. of dextrose, and is sterilized in the autoclave. To secure the best toxic product, the bacilli at hand must be carefully studied and that naturally possessing the strongest toxicogenic power employed for the cultures. The greatest toxicity seems to develop between the fifth and seventh days. If the culture is permitted to remain in the incubating oven beyond this period, the toxin gradually is transformed to toxoid and its activity declines. The fatal dose for a 250–300 gram guinea-pig should be about 0.001 c.c. given hypodermically.

II. The Immunization of the Animals.—All commercial manufacturers of diphtheria antitoxic serums now use horses, as recommended by Roux, instead of the sheep, dogs, and goats with which the earlier investigators worked. The horse is readily immunized,

* "Deutsche med. Wochenschrift," 1890, Nos. 49 and 50; "Zeitschrift für Hygiene," XII, 1, 1892.

† "Journal of Experimental Medicine," May and July, 1899, p. 373.

gives an abundant supply of blood which clots readily and yields a beautiful clear amber serum.

The horse selected should be in perfect health, and should be tested with mallein and tuberculin to avoid obscure glanders and tuberculosis.

A small dose of the toxic bouillon—say 0.1 c.c.—should be given in the beginning, as one occasionally finds exceptionally susceptible animals that will succumb to larger doses. If a marked local and general reaction follows, it may be better to try another animal. If no reaction is brought about, the immunization is carried on as rapidly as possible. The toxin is injected hypodermatically into the tissues of the neck, the skin being thoroughly cleaned and disinfected before each injection. The doses are cautiously increased and may often be doubled each day. If any unfavorable symptoms arise, treatment must be interrupted for a day or two. The animal yields good antitoxic serum when it can endure several doses of 500 c.c. of the strong toxin mentioned above.

III. Bleeding.—When the withdrawal of a small quantity of blood by a hypodermic needle introduced into the jugular vein shows that the serum contains a maximum antitoxic strength (300 to 1000 units per cubic centimeter), the horse is ready to bleed. Some horses can be bled without resistance, but most of them require to be fastened in appropriate stocks. The blood is taken from the jugular vein, which is superficial, of large size, and easily accessible. The skin is carefully shaved over an area about 9 square inches in extent, thoroughly disinfected. A small incision is made over the center of the vein, which is made prominent by pressure at the base of the neck, and the point of a small sterile trocar being inserted in the incision through the skin, it is directed obliquely upward into the vein. The blood is allowed to flow through a sterile tube attached to the cannula into sterile bottles prepared to receive it. A large horse may furnish 7 to 9 liters; small horses 5 to 7 liters.

IV. Preparation of the Serum.—The blood is stood away in a cool place until the clot retracts after coagulation and the clear serum separates. The serum is then withdrawn under strict aseptic precautions. It is variously prepared for the market. Some manufacturers bottle it without any added preservative; some add a crystal of thymol; some Pasteurize it; some add carbolic acid; some add trikresol.

The plain serum would be ideal, but the danger of subsequent contamination through careless treatment makes it rather better to have an antiseptic added. Trikresol is probably the most satisfactory of these, though it throws down a precipitate that necessitates the filtration of the product, and leaves the serum slightly opalescent.

V. Determining the Potency of the Serum.—The potency of the serum is expressed as so many "*immunizing units.*" Only one method of testing is produced at the present time, though to understand it, it seems wise to mention the original method from which it was derived.

(A) *Behring's Method.*—Behring's unit was an arbitrary standard chosen in consequence of certain conditions existing at the time it was devised. It is difficult to understand apart from the circumstances governing its creation, but may be defined as "*Ten times the least quantity of antitoxin serum that will protect a standard* (300 *gram*) *guinea-pig against ten times the least certainly fatal dose of toxic bouillon.*"

The method of determining it is not difficult to those skilled in laboratory technic, and is as follows:

1. Determine accurately the least certainly fatal dose of a sterile diphtheria toxic bouillon for a standard guinea-pig.

2. Determine accurately the least quantity of the serum that will

protect the guinea-pig against *ten times* the above determined least fatal dose of toxin.

3. Express the required dose of antitoxic serum as a fraction of a cubic centimeter and multiply by 10; the result is one unit.

Example: It is found that 0.01 c.c. of a toxic bouillon kills at least 9 out of 10 guinea-pigs, and is therefore the least certainly fatal dose. Guinea-pigs receive ten times this dose of the toxic bouillon plus varying quantities of the serum to be tested, measured by dilution—say $\frac{1}{2000}$ c.c., $\frac{1}{2500}$ c.c., $\frac{1}{3000}$ c.c. The first two live. The fraction $\frac{1}{2500}$ is now multiplied by 10; $\frac{1}{2500} \times 10 = \frac{1}{250} = 1$ unit. So we find that each cubic centimeter of the serum contains 250 units.

This method would be satisfactory were it not for certain variations in the toxic bouillon by which the strength is worked out. Ehrlich,* in an elaborate investigation of these changes, has clearly proved that an ever-changing toxin cannot be a satisfactory standard, because it does not possess uniform combining affinity for the antitoxin. He shows by a labored scheme that the toxicity of the bouillon is no index to its antitoxin-combining power, which, of course, must be the foundation of the test. The toxin, under natural conditions, is changed with varying rapidity into toxoids, of which he demonstrates three groups—prototoxoids, syntoxoids, and epitoxoids. The epitoxoids have a greater antitoxin-combining power than the toxin itself, yet have no toxic action upon the guinea-pigs, hence cause confusion in the results.

To secure a satisfactory measure of the antitoxic strength of a serum, it is therefore more important to first determine the antitoxin-combining power of the toxin or toxic bouillon to be used, than to determine its guinea-pig fatality, and this is what Ehrlich endeavors to do.

(B) *Ehrlich's Method.*—In this method the unit is the same as in Behring's method, but its determination is arrived at by a very important modification of the method, by which the standard of measurement is a special antitoxin of known strength, by which the antitoxin-combining power of the test toxic bouillon is first determined. Ehrlich began by determining the antitoxic value of a serum as accurately as possible by the old method and then used that serum as the standard for all further determinations. The serum was dried in a vacuum, and two grams of the dry powder were placed in each of a large number of small vacuum tubes, connecting with a small bulb of phosphoric anhydride. In this way the standard powder was protected from oxygen, water, and other injurious agents by which variations in its strength could be initiated. Periodically one of these tubes was opened and the contained powder dissolved in 200 c.c. of a mixture of 10 per cent. aqueous solution of sodium chloride and glycerin. The subsequent calculations are all based upon the strength of the antitoxin powder. In Ehrlich's first test serum 1 gram of the dry powder represented 1700 units. Of the solution mentioned, 1 c.c. represented 17 units; $\frac{1}{17}$ c.c., one unit.

Having by dilution—1 c.c. of the first dilution in 17 of water—secured the standard unit of antitoxin in a convenient bulk for the subsequent manipulations, it is mixed with varying quantities of the toxic bouillon to be used for testing the new serums, until the least quantity is determined that will cause the death of a 250-gram guinea-pig in exactly four days, when carefully injected beneath the skin of the animal's abdomen. This quantity of toxin is the *test dose.* If the toxic bouillon was "normal" in constitution, it should represent 100 of the least certainly fatal doses that formed the basis of the old

* "Klinisches Jahrbuch," 1897.

method of testing, but as toxic bouillons contain varying quantities of toxoids it may equal anywhere from fifty to one hundred and fifty times that dose.

The test dose of toxic bouillon, having been determined, remains invariable throughout the test as before, the serum to be tested for comparison with the standard being modified. The calculation is, however, different because the guinea-pig is receiving, not ten times, but more nearly one hundred times the least fatal dose, and the quantity of the antitoxic serum that preserves life beyond the fourth day is itself the unit.

Example: The sample of serum issued as the standard contains 17 units per cubic centimeter. Serum 1 c.c. + water 16 c.c. = 1 c.c. is the unit. 1 c.c. of the dilution containing one antitoxic unit is mixed with 0.01, 0.025, 0.05, 0.075, 0.1 c.c. of the toxic bouillon. All the animals receiving less than 0.1 c.c. live. A new series is started, and the guinea-pigs all weighing exactly 250 grams receive 1 unit of the antitoxin plus toxic bouillon 0.08, 0.09, 0.095, 0.097, 0.1, 0.11, 0.12, etc. It is found that all receiving more than 0.097 die in four days, but that the animal receiving that dose, though very ill, lives longer. The test dose may then be assumed to be 0.1, or it may be calculated more closely if desired.

To test the serum itself, guinea-pigs weighing exactly 250 grams are now all given toxic bouillon 0.1 c.c. plus varying quantities of the serum—$\frac{1}{200}$, $\frac{1}{300}$, $\frac{1}{400}$, etc. All live except those receiving less than $\frac{1}{400}$, which die about or on the fourth day. The serum can then be assumed to have 400 units per cubic centimeter unless it be desired to test more closely.

Standard test serums for making tests of antitoxic serums by the Ehrlich method were first shipped at small expense from the Kaiserliches Institut für Serum-Therapie at Höchst-on-the-Main, but at present the United States Public Health and Marine Hospital Service has undertaken the control of the manufacture of therapeutic serums and kindred products in the United States, issuing licenses to those engaged in legitimate manufacture, and also furnishing a standard serum, similar to that of Ehrlich, from their own laboratory in Washington.

A full description of "The Immunity Unit for Standardizing Diphtheria Antitoxin," by M. J. Rosenau, Director of the Hygienic Laboratory, can be found in Bulletin No. 21 of the U. S. Public Health and Marine Hospital Service, Washington, 1905.

As the quantity to be injected at each dose diminishes according to the number of units per cubic centimeter the serum contains, it is of the highest importance that therapeutic serums be as strong as possible. Various methods of concentration have been suggested, such as the partial evaporation of the serum *in vacuo*, but none has yet proved satisfactory. Bujwid* and H. C. Ernst† found that when an antitoxic serum is frozen and then thawed, it separates into two layers, the upper stratum watery, the lower yellowish, the antitoxic value of the yellowish layer being about three times that of the original serum, the upper layer consisting chiefly of water.

Administration.—Ehrlich asserts that a dose of 500 units is valueless for the treatment of diphtheria, 2000 units being

*"Centralbl. f. Bakt. u. Parasitenk.," Sept. 1897, Bd. XXII, Nos. 10 and 11, p. 287.

†"Jour. Boston Soc. of Med. Sci.," May, 1898, vol. II, No. 8, p. 137.

probably an average dose for an adult, and 1000 units for a child. As the remedy is practically harmless, it is far better to err on the side of administering too much than on that of not enough. Forty thousand units have been administered to a moribund child with resulting cure.

Diphtheria paralysis is more frequent after the use of antitoxin than in cases treated without it. In a paper upon this subject I * have shown that this is to be expected, as the palsies usually occur after bad cases of the disease, of which a far greater number recover when antitoxin is used for treatment.

An interesting collection of statistics upon the antitoxic treatment of diphtheria in the hospitals of the world has been published by Professor Welch,† who, excluding every possible error in the calculations, "shows an apparent reduction of case-mortality of 55.8 per cent."

Nothing should so impress the clinician as the necessity of beginning the antitoxin treatment *early in the disease.* Welch's statistics show that 1115 cases of diphtheria treated in the first three days of the disease yielded a fatality of 8.5 per cent., whereas 546 cases in which the antitoxin was first injected after the third day of the disease yielded a fatality of 27.8 per cent.

On the other hand, it can scarcely be said that any time is *too late* to begin the serum treatment, for the experiences of Burroughs and McCollum in the Boston City Hospital show that by the immediate and repeated administration of very large doses of the serum, apparently hopeless cases, far advanced in the disease, may often be saved.

After the toxin has occasioned destructive organic lesions of the nervous system and in the various organs and tissues of the body, no amount of neutralization can restore the integrity of the parts, and in such cases antitoxin must fail.

Urticaria and erythema sometimes follow the injection of antitoxic serum, for reasons not clearly understood. In a few cases pains in the bones and joints have been complained of. The occurrence of tetanus following the employment of serum drawn from a horse suffering from tetanus was observed in a number of cases in St. Louis. In rare cases

* "Medical Record," New York, 1897.

† "Bull. of the Johns Hopkins Hospital," July and Aug., 1895.

in which local and metastatic abscesses have been observed, the condition is probably correctly attributable to infection from the patient's skin or from the syringe.

I have found that the serums are by no means regular in the rapidity of deterioration, so that no very old serum should be used.

Freezing is without effect upon the serum and ordinary temperature-changes are harmless to it. The antitoxic power is destroyed at 60° C., the point at which the serum coagulates. The antitoxin is precipitated with the globulins.*

The serums from different horses probably vary much in both their irritant and globulicidal properties, so that mixed serums from a number of horses may be preferable to that from a single horse.

Prophylaxis.—The serum can be relied upon for prophylaxis in cases of exposure to diphtheria infection. In most cases a single dose of 500 units is sufficient for the purpose. The transitory nature of the immunity afforded by prophylactic injections of antitoxin is probably dependent upon the fact that the antitoxin is slowly excreted through the kidneys.

Treatment.—For treatment no dose smaller than 1000 units should be given and in older children and adults the dose should be 2000 units. The administration of the remedy should be repeated in twelve hours if the disease is one or two days old, in six hours if three or four days old, in four hours if still older. The serum may have to be given two, three, four, or even more times, according to the case.

Diphtheria antitoxin is always to be administered by the hypodermic method, wherever the skin is loose. Some clinicians prefer to inject into the abdominal wall, some into the tissues of the back. A slightly painful swelling is formed, which usually disappears in a short time. Occasionally there is an immediate outbreak of local urticaria —rarely general urticaria. Sometimes considerable local erythema results. The occasional reports of successful oral administration are very doubtful observations. Serums of high unit strength can be given with the ordinary hypodermic syringe; those of lower strength, of which a larger quantity is required, must be given with

* See paper by J. P. Atkinson, "Journal of Experimental Medicine," Sept. and Nov., 1899, vol. IV, Nos. 5 and 6.

a special "antitoxin syringe." The syringe should always be carefully sterilized by boiling, and the packings, etc., found to be in good condition before it is filled with antitoxin.

Bacilli Resembling the Diphtheria Bacillus.

The *pseudo-diphtheria bacillus* (bacillus of Hoffmann*)—Bacillus pseudo-diphthericus—was first found by Löffler† in diphtheria pseudo-membranes and in the healthy mouth and pharynx. It is also found upon the conjunctiva, especially in xerosis conjunctivæ, and corresponds to the so-called Bacillus xerosis conjunctivæ, described by Kutschbert and Neisser‡ as the cause of xerosis, but now recognized as a constant inhabitant of the conjunctiva in health

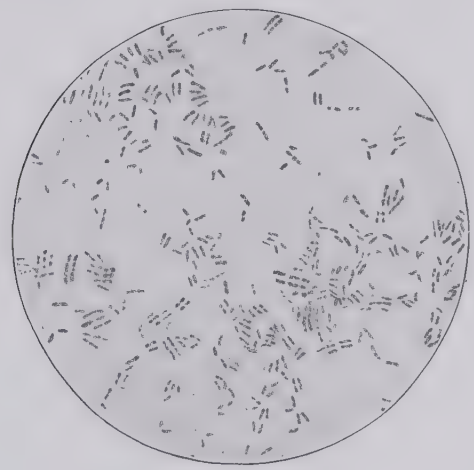


Fig. 128.—Pseudo-diphtheria bacilli (Muir and Ritchie).

and disease. By some authors this bacillus is thought to cause chronic ulcerative keratitis and chalazion. The pseudo-diphtheria bacillus is also found in the nose and upon the skin, where it usually associates itself with Staphylococcus aureus. It has been found in impetigo, acne, and variola pustules, and it has also at times been isolated from

*"Wiener klin. Woch.," 1888, No. 3.
†"Centralbl. f. Bakt. u. Parasitenk.," II, 105.
‡"Deutsche med. Woch.," 1884, No. 24.

the internal organs, as in the cases of Egyptian dysentery studied by Kruse and Pasquale.* Ohlmacher has also found it with other bacteria in pneumonia; Babes, in gangrene of the lung; and Howard,† in a case of ulcerative endocarditis not succeeding diphtheria.

While various authors have endeavored to point out morphologic and cultural differences by which the diphtheria and pseudo-diphtheria bacilli can be differentiated, it must be admitted that the variations of the latter organism are so numerous that all rules fail. The only criterion for specific differentiation is the ability of the true diphtheria bacillus to form toxin, which the pseudo-diphtheria bacillus entirely lacks. The inoculation of the pseudo-diphtheria bacillus into animals is followed by no pathologic changes.

G. F. Petri‡ found no substances in filtrates of cultures of Hoffmann's bacillus capable of neutralizing diphtheria antitoxin; he also found that horses immunized with large quantities of filtrates of the Hoffmann bacillus did not produce any antitoxin to diphtheria toxin. Eleven different cultures were studied and the results are very important.

Knapp§ shows that there is a chemico-biological difference between the true and pseudo-diphtheria bacilli, in that the pseudo-bacillus ferments saccharose, which the true bacillus does not, and that the true bacillus ferments dextrin, which the pseudo-bacillus does not.

Park‖ carefully studied this subject, and found that all bacilli with the typical morphology of the diphtheria bacillus, found in the human throat, are virulent Klebs-Löffler bacilli, while forms found in the throat closely resembling them, but more uniform in size and shape, shorter in length, and of more homogeneous staining properties with Löffler's alkaline methylene-blue solution, can with reasonable safety be regarded as pseudo-diphtheria bacilli, especially if it be found that they produce an alkaline rather than an acid reaction by their growth in bouillon. The pseudo-diphtheria bacilli were found in about 1 per cent. of throats ex-

* "Zeitschrift für Hygiene," XVI, 1.

† "Bull. of the Johns Hopkins Hospital," 1893, 30.

‡ "Jour. of Hygiene," vol. v, No. 2, April, 1905, p. 134.

§ "Jour. of Med. Research," XI, 1904, p. 475.

‖ "Scientific Bulletin No. 1," Health Department, city of New York, 1895.

amined in New York; they seem to have no relationship to diphtheria, and are never virulent.

Dr. Alice Hamilton* carefully studied 29 organisms, of which 26 corresponded fully with the pseudo-diphtheria bacilli. They were divisible into three groups: I, Those non-pathogenic for guinea-pigs; II, those that produce general bacteremia in guinea-pigs, and are neutralized by treatment with the serum of a rabbit immunized against a member of the group; III, organisms which form gas in glucose media, produce bacteremia in guinea-pigs, and are neutralized neither by diphtheria antitoxin, nor by pseudo-diphtheria. Some of the organisms of the second group are also pathogenic for man. Instead of regarding the pseudo-diphtheria bacillus as a harmless saprophyte, Dr. Hamilton believes it an important organism explaining some of the paradoxes that we find at hand. Thus, cases of supposed diphtheria irremediable by or deleteriously affected by antitoxic serum may depend upon one of these organisms. It is also probably one of them that Councilman found in his case of "general infection by B. diphtheriæ," and that Howard encountered in his case of acute ulcerative endocarditis without diphtheria, from the valves of whose heart cultures of a diphtheria-like organism not pathogenic for guinea-pigs were isolated.

The observation of Martini,† that the diphtheria bacillus will not grow in fluid antitoxic serum in which the pseudo-diphtheria bacillus thrives, I have not been able to confirm.

Having practically the same cultural and staining reactions as the diphtheria bacillus, the question presents itself, Is the pseudo-diphtheria bacillus the diphtheria bacillus in an attenuated condition? This question we are, as yet, unable to answer. Every attempt to bring back virulence to the pseudo-bacilli has failed, and we know it only as a saprophyte.

*"Jour. Infectious Diseases," 1904, I, p. 690.

†"Centralbl. f. Bakt. u. Parasitenk.," Jan. 30, 1897, Bd. XXI, No. 3.

CHAPTER IV.

CHOLERA AND SPIRILLA RESEMBLING THE CHOLERA SPIRILLUM.

Spirillum Choleræ Asiaticæ (Koch*).

General Characteristics.—A motile, flagellated, non-sporogenous, liquefying, non-chromogenic, parasitic and saprophytic, pathogenic, aerobic and optionally anaerobic spirillum, staining by ordinary methods, but not by Gram's method.

Cholera is a disease endemic in certain parts of India and probably indigenous in that country. Though early mention of it was made in the letters of travelers, and though it appeared in medical literature and in governmental statistics more than a century ago, we find that little attention was paid to the disease, except in its disastrous effect upon the armies, native and European, of India and adjacent countries. The opening up of India by Great Britain in the last half century made scientific observation of the disease possible and permitted us to determine the relation its epidemics bear to the manners and customs of the people.

The filthy habits of the Oriental people, their poverty, crowded condition, and peculiar religious customs, are all found to aid in the distribution of the disease. Thus, the city of Benares drains into the Ganges River by a most imperfect system, which distributes the greater part of the sewage immediately below the banks upon which the city is built and along which are the numerous "Ghats" or staircases by which the people reach the sacred waters. It is a matter of religious observance for every zealot who makes a pilgrimage to the "sacred city" to take a bath in and drink a quantity of this sacred but polluted water, and it may be imagined that the number of pious Hindoos who leave Benares with "comma bacilli" in their intestines

* "Deutsche med. Wochenschrift," 1884–1885, Nos. 19, 20, 37, 38, and 39.

or upon their clothes must be great, for there are few months in the year when the city is exempt from cholera.

The pilgrimages and great festivals of the Hindoos and Moslems, by bringing together enormous numbers of people to crowd in close quarters where filth and bad diet prevail, cause a rapid increase in the number of cases during these periods and facilitate the distribution of the disease when the festivals break up. Probably no more favorable conditions for the dissemination of a disease can be imagined than occurs with the return of the Moslem pilgrims from Mecca. The disease extends readily along the regular lines of travel, visiting town after town, until from Asia it has frequently extended into Europe, and by steamships plying foreign waters has several times been carried to our own continent. Many cases are on record which show conclusively how a single ship, having a few cholera cases on board, may be the starting-point of an outbreak of the disease in the port at which it arrives.

Specific Organism.—The discovery of the spirillum of cholera was made by Koch, who was appointed one of a German commission appointed to study the disease in Egypt and India in 1883–84. Since his discovery and published investigations, the spirillum has been subjected to much careful investigation, and an immense amount of literature, a large part of which was stimulated by the Hamburg epidemic of a few years ago, has accumulated.

Distribution.—The cholera spirilla can be found with great regularity in the intestinal evacuations of cholera cases, and can often be found in drinking-water and milk, and upon vegetables, etc., in cholera-infected districts. There can be little doubt that they find their way into the body with the food and drink. Cases in the literature show how cholera germs enter drinking-water and are thus distributed; how they are sometimes thoughtlessly sprinkled over green vegetables offered for sale in the streets, with infected water from polluted gutters; how they enter milk with water used to dilute it; how they appear to be carried about in clothing and upon food-stuffs; how they can be brought to articles of food by flies that have preyed upon cholera excrement; and other interesting modes of infection. The literature is so vast that it is scarcely possible to mention even the most instructive examples. A bacteriologist became infected while experimenting with the cholera spirilla

in Koch's laboratory. It is commonly supposed that the cholera organism may remain alive in water for an almost unlimited length of time, but experiments have not shown this to be the case. Thus, Wolffhügel and Riedel have shown that if the spirilla be planted in sterilized water they grow with great rapidity after a short time, and can be found alive after months have passed. Fränkel, however, points out that this ability to grow and remain vital for long periods in sterilized water does not guarantee the same power of growth in unsterilized water, for in the latter the simultaneous growth of other bacteria serves to extinguish the cholera spirilla in a few days.

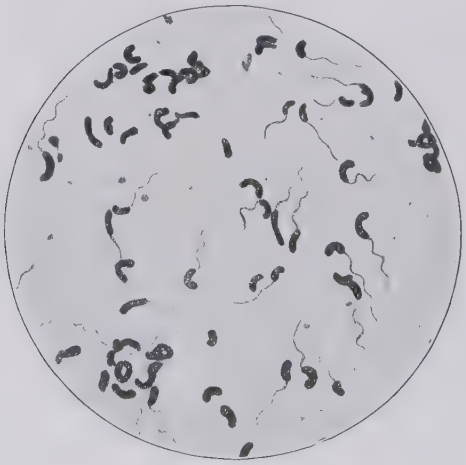


Fig. 129.—Cholera spirilla (Muir and Ritchie).

Morphology.—The micro-organism described by Koch, and now generally accepted to be the cause of cholera, is a short rod about half the length of a tubercle bacillus, considerably stouter, with rounded ends, and a distinct curve, so that the original name by which it was known, the "comma bacillus," applies very well (Figs. 129, 130).

A study of the growth of the organism and the forms which it assumes upon different culture media soon convinces us that we have to do with an organism in no way related to the bacilli. When the conditions of nutrition are good, multiplication by fission progresses with rapidity; but when adverse conditions arise, long spiral threads—unmistakable spirilla—develop. Fränkel found that the

exposure of the cultures to unusually high temperatures, the addition of small amounts of alcohol to the culture media, and other unfavorable conditions favored the production of spirals instead of "commas." One of the most common forms is that in which two short curved individuals are conjoined in an S-shaped curve.

The cholera spirilla are actively motile, and in hanging-drop preparations can be seen to swim about with great rapidity. Both comma-shaped and spiral organisms move with a rapid rotary motion.

The presence of flagella can be demonstrated without

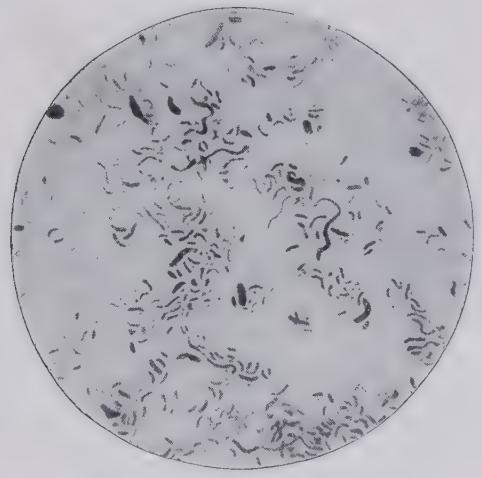


Fig. 130.—Spirillum of Asiatic cholera, from a bouillon culture three weeks old, showing long spirals. × 1000 (Fränkel and Pfeiffer).

difficulty. Each spirillum possesses a single flagellum attached to one end (spiromonas).

Involution-forms of bizarre appearance are common in old cultures of the spirillum, and sometimes in fresh cultures many individuals show by granular cytoplasm and irregular outline that they are degenerated. Cholera spirilla from various sources differ in the extent of involution.

In partially degenerated cultures containing long spirals Hüppe observed, by examination in the "hanging-drop," certain large spheric bodies which he described as spores (arthrospores). Koch and, indeed, all other observers fail

to find spores in the cholera organism, and the nature of the bodies described by Hüppe must be regarded as doubtful.

Staining.—The cholera spirillum stains well with the ordinary aqueous solutions of the anilin dyes, especially fuchsin. At times the staining must be continued for from five to ten minutes to secure homogeneity. The organism does not stain by Gram's method. It may be colored and examined while alive; thus, Cornil and Babes, in demonstrating it in the rice-water discharges, "spread out one of the white mucous fragments upon a glass slide and allow it to dry partially; a small quantity of an exceedingly

Fig. 131.—Cover-glass preparation of a mucous floccule in Asiatic cholera. × 650 (Vierordt).

weak solution of methyl violet in distilled water is then applied to it, and it is flattened out by pressing down a cover-glass, over which is placed a fragment of filter paper, which absorbs any excess of fluid at the margin of the cover-glass. The characteristics of comma bacilli so prepared and examined with an oil-immersion lens (× 700–800) are readily made out because of the slight stain they take up, and because they still retain the power of vigorous movement, which would be entirely lost if the specimen were dried, stained, and mounted in the ordinary fashion."

Isolation of the Organism.—One of the best methods of securing a pure culture of the cholera spirillum, and also

of making a bacteriologic diagnosis of the disease in a suspected case, is probably that of Schottelius.

A small quantity of the fecal matter is mixed with bouillon and stood in an incubating oven for twenty-four hours. If the cholera spirilla are present they will grow most rapidly at the surface of the liquid when the supply of air is good. A pellicle will be formed, a drop from which, diluted in melted gelatin and poured upon plates, will show typical colonies.

Cultivation.—The cholera organism is easily cultivated, and grows luxuriantly upon the usual laboratory media.

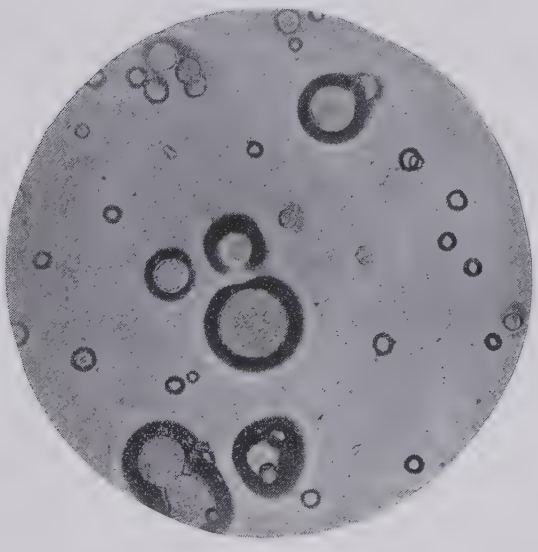


Fig. 132.—Spirillum of Asiatic cholera; colonies two days old upon a gelatin plate. × 35 (Heim).

Colonies.—The colonies grown upon gelatin plates are characteristic and appear in the lower strata of the gelatin as small white dots, which gradually grow out to the surface, effect a slow liquefaction of the medium, and then appear to be situated in little pits with sloping sides (Fig. 132). This appearance suggests that the plate is full of little holes or air-bubbles, and is due to the slow evaporation of the liquefied gelatin.

Under the microscope the colony of the cholera spirillum is fairly well characterized. The little colonies that have

not yet reached the surface of the gelatin soon show a pale yellow color and an irregular contour. They are coarsely granular, the largest granules being in the center. As the colony increases in size the granules do the same and attain a peculiar transparent character suggestive of powdered glass. The slow liquefaction causes the colony to be surrounded by a transparent halo. As the liquefied gelatin evaporates, the colony begins to sink, and also to take on a peculiar rosy color.

Gelatin.—In puncture cultures in gelatin the growth is

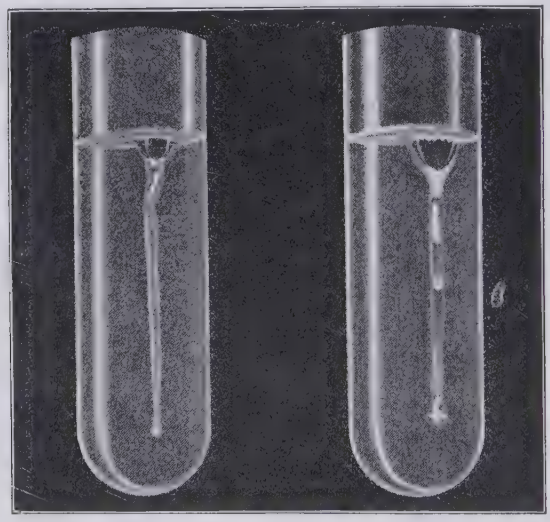


Fig. 133.—Spirillum choleræ asiaticæ; gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

again quite characteristic (Fig. 133). It occurs along the entire puncture, best at the surface, where it is in contact with the atmosphere. Liquefaction of the medium begins almost at once, keeps pace with the growth, but is always more marked at the surface than lower down. The result of this is the formation of a short, rather wide funnel at the top of the puncture. As the growth continues, evaporation of the medium takes place slowly, so that the liquefied gelatin is lower than the solid surrounding portions, and the growth appears to be surmounted by an air-bubble.

The luxuriant development of the spirilla in the liquefying

gelatin is followed by the formation of considerable sediment in the lower third or half of the liquefied area. This solid material consists of masses of spirilla which have probably completed their life-cycle and become inactive. Under the microscope they exhibit the most varied involution-forms. The liquefaction reaches the sides of the tube in from five to seven days, but is not complete for several weeks.

Agar-agar.—When planted upon the surface of agar-agar the spirilla produce a grayish-white, shining, translucent growth along the entire line of inoculation. It is in no way peculiar or characteristic. The vitality of the organism is retained much better upon agar-agar than upon gelatin, and, according to Fränkel, the organism can be transplanted and grown when nine months old.

Blood-serum.—The growth upon blood-serum is also without distinct peculiarities; gradual liquefaction of the medium occurs.

Potato.—Upon potato the spirilla grow well, even when the reaction is acid. In the incubator, at a temperature of 37° C., a transparent, slightly brownish or yellowish-brown growth, somewhat resembling that of glanders, is produced. It contains large numbers of long spirals.

Bouillon.—In bouillon and in peptone solution the cholera organisms grow well, especially upon the surface, where a folded, wrinkled pellicle is formed, the culture fluid remaining clear.

Milk.—In milk the growth is luxuriant, but does not visibly alter its appearance. The existence of cholera organisms in milk is, however, rather short-lived, for the occurrence of acidity at once destroys them.

Vital Resistance.—Although an organism that multiplies with great rapidity under proper conditions, the cholera spirillum does not possess much resisting power. Sternberg found that it was killed by exposure to 52° C. for four minutes, but Kitasato found that ten or fifteen minutes' exposure to 55° C. was not always fatal to it. In a moist condition the organism may retain its vitality for months, but it is very quickly destroyed by desiccation, as was found by Koch, who observed that when dried in a thin film its power to grow was destroyed in a few hours. Kitasato found that upon silk threads the vitality might be retained longer. Abel and Claussen * have shown that it

* "Centralbl. f. Bakt. u. Parasitenk.," Jan. 31, 1895, vol. xvii, No. 4.

does not live longer than twenty or thirty days in fecal matter, and often disappears in from one to three days. The organism is very susceptible to the influence of carbolic acid, bichlorid of mercury, and other germicides, and is also destroyed by acids. Hashimoto * found that it could not live longer than fifteen minutes in vinegar containing 2.2–3.2 per cent. of acetic acid.

According to Fränkel, in eight weeks the organisms in the liquefied cultures all die, and cannot be transplanted. Kitasato, however, has found them living and active on agar-agar after from ten to thirty days, and Koch was able to demonstrate their vitality after two years.

This low vital resistance of the microbe is very fortunate, for it enables us to establish satisfactory quarantine for the prevention of the spread of the disease. Excreta, soiled clothing, etc., are readily rendered harmless by the proper use of disinfectants. Water and food are rendered innocuous by boiling or cooking. Vessels may be disinfected by thorough washing with jets of boiling water discharged through a hose connected with a boiler, and baggage can be sterilized by superheated steam.

Metabolic Products.—Indol is one of the characteristic metabolic products of the cholera spirillum. As the cholera organisms also produce nitrites, all that is necessary to demonstrate its presence in a colorless solution is to add a drop or two of chemically pure sulphuric acid, when the well-known reddish color will appear.

The organism also produces acid in milk and other media. Bitter has also shown that the cholera organism produces a peptonizing and probably also a diastatic ferment.

Toxic Products.—Rietsch thinks the intestinal changes depend upon the action of the peptonizing ferment. Cantani, Nicati and Rietsch, Van Ermengem, Klebs, and others found toxic effects from cultures administered to dogs and other animals. Several toxic metabolic products of the spirilla have been isolated. Brieger, † Brieger and Fränkel, ‡ Gamaléia, § Sobernheim, ‖ and Villiers have

* "Kwai Med. Jour.," Tokyo, 1893.

† "Berliner klin. Wochenschrift," 1887, p. 817.

‡ "Untersuchungen über die Bakteriengifte," etc., Berlin, 1890.

§ "Archiv de Méd. exp.," IV, No. 2.

‖ "Zeitschrift für Hygiene," XIV, 145, 1893.

studied more or less similar toxic products. The real toxic substance is, however, not known.

Pathogenesis.—Through what activity the cholera organism provokes its pathogenic action is not yet determined. The organisms, however, abound in the intestinal contents, penetrate sparingly into the tissues, but slightly invade the lymphatics, and almost never enter the circulation; hence it is but natural to conclude that the first action must be an irritative one depending upon toxin-formation in the intestine.

In the beginning of the disease the small and large intestines are deeply congested, almost velvety in appearance, and contain liquid fecal matter. The patient suffers from diarrhea, by which the feces are hurried on and become extremely thin from the admixture of a copious watery exudate. As the feces are hurried out, more and more of the aqueous exudate accumulates, until the intestine seems to contain only watery fluid. The solitary glands and Peyer's patches are found enlarged and the mucosa becomes macerated and necrotic, its epithelium separating in small shreds or flakes. The evacuations of watery exudate rich in these shreds constitute the characteristic "rice-water discharges" of the disease. As the disease progresses, the denudation of tissue results in the formation of good-sized ulcerations. Perforations and deep ulcerations are rare. Pseudo-membranous formations not infrequently occur upon the abraded and ulcerated surfaces. The other mucous membranes of the alimentary apparatus become congested and abraded; the parenchyma of the liver, kidneys, and other organs becomes markedly degenerated, so that the urine becomes highly albuminous and very scanty in consequence of the anhydremia. The cardio-vascular, nervous, and respiratory systems present no characteristic changes.

Intraperitoneal injection of the virulent cultures produces fatal peritonitis in guinea-pigs.

Supposing that the lower animals were immune against cholera because of the acidity of the gastric juice, Nicati and Rietsch, Van Ermengem, and Koch have suggested methods by which the micro-organisms can be introduced directly into the intestine. The first-named investigators ligated the common bile-duct of guinea-pigs, and then injected the spirilla into the duodenum with a hypodermic

needle, with the result that the animals usually died, sometimes with choleraic symptoms. The excessively grave nature of the operation upon such a small and delicately constituted animal as a guinea-pig, however, greatly lessens the value of the experiment. Koch's method of infection by the mouth is much more satisfactory. By injecting laudanum into the abdominal cavity of guinea-pigs the peristaltic movements of the intestine can be checked. The amount necessary for the purpose is large and amounts to about 1 gram for each 200 grams of body-weight. It completely narcotizes the animals for a short time (one to two hours), but they recover without injury. The contents of the stomach are neutralized after administering the opium, by introducing 5 c.c. of a 5 per cent. aqueous solution of sodium carbonate through a pharyngeal catheter. With the gastric contents thus alkalinized and the peristalsis paralyzed, a bouillon culture of the cholera spirillum is introduced through the stomach-tube. The animal recovers from the manipulation, but shows an indisposition to eat, is soon observed to be weak in the posterior extremities, subsequently is paralyzed, and dies within forty-eight hours. The autopsy shows the intestine congested and filled with a watery fluid rich in spirilla—an appearance which Fränkel declares to be exactly that of cholera. In man, as well as in these artificially infected animals, *the spirilla are never found in the blood or tissues*, but only in the intestine, where they frequently enter between the basement membrane and the epithelial cells, and aid in the detachment of the latter.

Issaëff and Kolle found that when virulent cholera spirilla are injected into the ear-veins of young rabbits the animals die on the following day with symptoms resembling the algid state of human cholera. The autopsy in these cases showed local lesions of the small intestine very similar to those observed in cholera in man.

Guinea-pigs are also susceptible to intraperitoneal injections of the spirillum, and speedily succumb. The symptoms are rapid fall of temperature, tenderness over the abdomen, and collapse. The autopsy shows an abundant fluid exudate containing the micro-organisms, and injection and redness of the peritoneum and viscera.

Specificity.—The cholera spirillum is present in the dejecta of cholera with great regularity, and as regularly absent

from the dejecta of healthy individuals and those suffering from other diseases. There is no satisfactory proof of the specific nature of the organisms to be obtained by experimentation upon animals. Animals are never affected by any disease similar to cholera during epidemics, nor do foods mixed with cholera discharges or with pure cultures of the cholera spirillum affect them. Subcutaneous inoculations do not produce cholera.

Detection of the Organism.—It often becomes a matter of importance to detect the cholera spirilla in drinking-water, and, as the number in which the bacteria exist in such a liquid may be very small, difficulty may be experienced in finding them by ordinary methods. One of the most expeditious methods is that recommended by Löffler, who adds 200 c.c. of the water to be examined to 10 c.c. of bouillon, allows the mixture to stand in an incubator for from twelve to twenty-four hours, and then makes plate cultures from the superficial layer of the liquid, where, if present, the development of the spirilla will be most rapid because of the free access of air. A similar method, suggested by Schottelius (see page 451), can be used to detect the spirilla in feces.

Immunity.—Gruber and Wiener, * Haffkine, † Pawlowsky, ‡ and Pfeiffer § have immunized animals against toxic substances from cholera cultures or against living cultures. There seems, according to the researches of Pfeiffer, to be no doubt that a protective substance exists in the blood of immunized animals. In the peritoneal infection of guinea-pigs the spirilla grow vigorously in the peritoneal cavity, and can be found in immense numbers after from twelve to twenty-four hours. If, however, together with the culture used for inoculation, a few drops of the serum from an immunized animal be introduced, Pfeiffer found that, instead of multiplying, the organisms underwent a peculiar granular degeneration and disappeared, the unprotected animal dying, the protected animal remaining well. This bacteriolytic change takes place as well in the test-tube as in the peritoneal cavity.

* "Centralbl. f. Bakt.," etc., 1892, XIV, p. 76.

† "Le Bull. méd.," 1892, p. 1113, and "Brit. Med. Jour.," 1893, p. 278.

‡ "Deutsche med. Wochenschrift," 1893, No. 22.

§ "Zeitschrift für Hygiene," Bd. XVIII and XX.

For a long time this bacterial destruction, known as "Pfeiffer's phenomenon," could not be explained. If the spirilla were placed in the immune serum, they were not destroyed; that the serum of the infected animal did not destroy them was evident enough from the existing choleraic peritonitis which would progress to a fatal termination if not checked by the serum, yet the combination of the serum of the infected animal and the immune serum sufficed to bring about bacteriolysis. At present the matter is fairly clear. We now know that the failure of the animal's own serum to destroy the bacteria probably depends upon insufficient immune body. The immune body contained in the immune serum could not produce any effect unless in the presence of an additional amount of complementary substance.

When the inflammatory exudate of the infected guinea-pig and the immune serum were combined, however, the one furnished the necessary immune body, the other the necessary complementary body, and the alexins were permitted to act upon the bacteria which were immediately destroyed and dissolved.

Pfeiffer and Vogedes * have applied this "immunity reaction" to the differentiation of cholera spirilla in cultures. A hanging drop of a 1 : 50 mixture of a powerful anticholera serum and a particle of cholera culture is made and examined under the microscope. The cholera spirilla at once become inactive, and are in a short time converted into little rolled-up masses. If the culture added be a spirillum other than the true cholera spirillum, instead of being destroyed the micro-organisms multiply and thrive in the mixture of serum and bouillon.

Sobernheim † found the Pfeiffer reaction specific against cholera alone, and thought the protection not due to the strongly bactericidal property of the serum, but to its stimulating effect upon the body-cells; for if the serum be heated to 60°–70° C., and its bactericidal power thus destroyed, it is still capable of producing immunity. This, of course, is in keeping with our present knowledge of the *immune body*, which is not destroyed by such temperatures.

The immunity produced by the injection of the spirilla

* "Centralbl. f. Bakt. u. Parasitenk.," March 21, 1896, Bd. XIX, No. 11.

† "Zeitschrift für Hygiene," XX, p. 438.

into guinea-pigs continues in some cases as long as four and a half months, but the power of their serum to confer immunity is lost much sooner.

Serum Therapy and Prophylaxis.—Of the numerous attempts to produce immunity against cholera in man or to cure cholera when once established in the human organism, nothing very favorable can be said. Experiments in this field are not new. As early as 1885 Ferrán, in Spain, administered hypodermic injections of pure virulent cultures of the cholera spirillum, in the hope of bringing about immunity. The work of Haffkine,* however, is the chief important contribution, and his method seems to be followed by a positive diminution of mortality in protected individuals. Haffkine uses two vaccines—one mild, the other so powerful that it would bring about extensive tissue-necrosis and perhaps death if used alone. His studies embrace more than 40,000 inoculations performed in India. The following extract will show results obtained in 1895:

"1. In all those instances where cholera has made a large number of victims,—that is to say, where it has spread sufficiently to make it probable that the whole population, inoculated and uninoculated, were equally exposed to the infection,—in all these places the results appeared favorable to inoculation.

"2. The treatment applied after an epidemic actually breaks out tends to reduce the mortality even during the time which is claimed for producing the full effect of the operation. In the Goya Garl, where weak doses of a relatively weak vaccine had been applied, this reduction was to half the number of deaths; in the coolies of the Assam-Burmah survey party, where, as far as I can gather from my preliminary information, strong doses have been applied, the number of deaths was reduced to one-seventh. This fact would justify the application of the method independently of the question as to the exact length of time during which the effect of this vaccination lasts.

"3. In Lucknow, where the experiment was made on small doses of weak vaccines, a difference in cases and deaths was still noticeable in favor of the inoculated fourteen to fifteen months after vaccination in an epidemic of exceptional virulence. This makes it probable that a protective effect could be obtained even for long periods of time if larger doses of a stronger vaccine were used.

"4. The best results seem to be obtained from application of middle doses of both anticholera vaccines, the second one being kept at the highest possible degree of virulence obtainable.

"5. The most prolonged observations on the effect of middle doses were made in Calcutta, where the mortality from the eleventh up to the four hundred and fifty-ninth day after vaccination was, among the inoculated, 17.24 times smaller, and the number of cases 19.27 times smaller than among the not inoculated."

* "Le Bull. méd.," 1892, p. 1113; "Indian Med. Gazette," 1893, p. 97; "Brit. Med. Jour.," 1893, p. 278.

Pawlowsky and others have found the dog susceptible to cholera, and have utilized it in the preparation of an antitoxic serum. The dogs were first immunized against attenuated cultures, then against more and more virulent cultures, until a serum was obtained whose value was estimated at 1 : 130,000 upon experimental animals.

Freymuth * and others have endeavored to secure favorable results from the injection of blood-serum from convalescent patients into the diseased. One recovery out of three cases treated is recorded.

In all these preliminaries the foreshadowing of a future therapeusis must be evident, but as yet nothing satisfactory has been achieved.

One of the chief errors made in the experimental preparation of anticholera serums is that efforts have been directed toward endowing the blood with the power of resisting and destroying the bacteria that rarely, if ever, reach it. The two essentials to be aimed at are an *antitoxin* to neutralize the depressing effects of the toxalbumin, and some means of destroying the bacteria in the intestine.

The cholera spirillum is one of a considerable-sized group of closely related organisms, from some of which it is differentiated with difficulty.

SPIRILLA RESEMBLING THE CHOLERA SPIRILLUM.

The Finkler and Prior Spirillum (Vibrio Proteus).

Similar in morphology to the spirillum of cholera, and in other respects closely related to it, is the spirillum obtained from the feces of a case of cholera nostras by Finkler and Prior † in 1884.

Morphology.—It is shorter and stouter, with a more pronounced curve than the cholera spirillum, and rarely forms long spirals. The central portion is also somewhat thinner than the ends, which are a little pointed and give the organism a less uniform appearance (Fig. 134). Involution forms are common in cultures, and appear as spheres, spindles, clubs, etc. Like the cholera spirillum,

* "Deutsche med. Wochenschrift," 1893, No. 43.

† "Centralbl. für allg. Gesundheitspflege," Bd. 1, Bonn, 1885; "Deutsche med. Wochenschrift," 1884, p. 632.

each organism is provided with a single flagellum situated at its end, and is actively motile. Although at first thought to be a variety of the cholera spirillum, marked differences of growth were soon observed, and showed the organism to be a separate species.

Staining.—The organism stains readily with the ordinary solutions, but not by Gram's method.

Cultivation.—Colonies. — The growth upon gelatin plates is rapid, and leads to such extensive liquefaction that four or five dilutions must frequently be made to secure few enough organisms to enable one to observe the

Fig. 134.—Spirillum of Finkler and Prior, from an agar-agar culture. × 1000 (Itzerott and Niemann).

growth of a single colony. To the naked eye the deep colonies appear as small white points (Fig. 135). They rapidly reach the surface, begin liquefaction of the gelatin, and by the second day appear about the size of lentils, and are situated in little depressions. Under the microscope they are yellowish-brown, finely granular, and are surrounded by a zone of sharply circumscribed liquefied gelatin. Careful examination with a high-power lens shows rapid movement of the granules in the colony.

Gelatin Punctures.—In gelatin punctures the growth takes place rapidly along the whole length of the puncture, forming a stocking-shaped liquefaction filled with cloudy

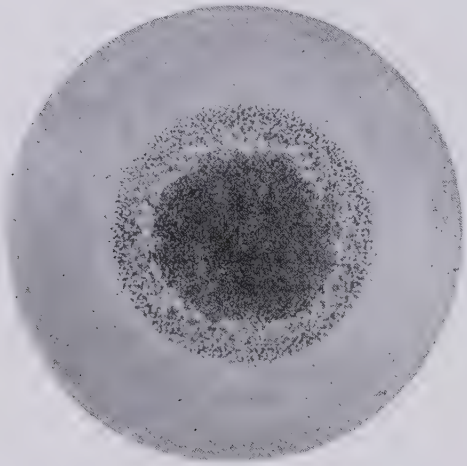


Fig. 135.—Spirillum of Finkler and Prior; colony twenty-four hours old, as seen upon a gelatin plate. × 100 (Fränkel and Pfeiffer).

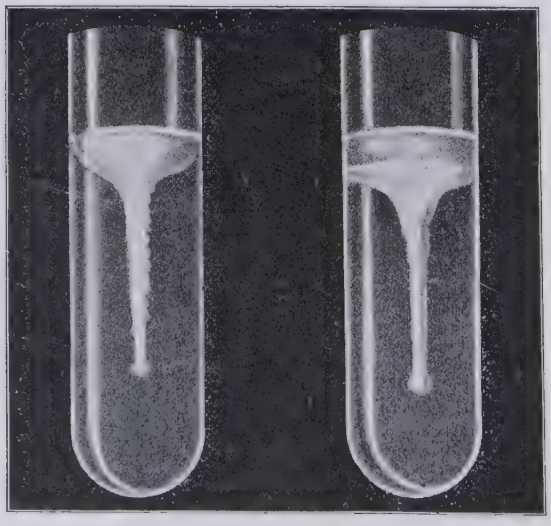


Fig. 136.—Spirillum of Finkler and Prior; gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

fluid which does not precipitate rapidly; a rather smeary, whitish scum is usually formed upon the surface. The more extensive and more rapid the liquefaction of the medium, the wider the top to the funnel, the absence of the air-bubble, and the clouded nature of the liquefied material, all serve to differentiate the culture from the cholera spirillum.

Agar-agar.—Upon agar-agar the growth is also rapid, and in a short time the whole surface of the culture medium is covered with a moist, thick, slimy coating, which may have a slightly yellowish tinge.

Bouillon.—In bouillon the organism causes a diffuse turbidity with a more or less distinct pellicle on the surface. In sugar-containing culture media it causes no fermentation and generates no gas.

Potato.—The cultures upon potato are also different from those of the cholera organism, for the Finkler and Prior spirilla grow rapidly at the room temperature, and produce a grayish-yellow, slimy, shining layer, which may cover the whole of the culture medium.

Blood-serum.—Blood-serum is rapidly liquefied by the organism.

The spirillum does not grow well in milk, and speedily dies in water.

Metabolic Products.—The organism does not produce indol. Buchner has shown that in media containing some glucose an acid reaction is produced. Proteolytic enzymes capable of dissolving gelatin, blood-serum, and casein are formed.

Pathogenesis.—It was at first supposed that if not the spirillum of cholera itself, this was a very closely allied organism. Later it was supposed to be the cause of cholera nostras. At present it is a question whether the organism has any pathologic significance. It was in one case secured by Knisl from the feces of a suicide, and has been found in carious teeth by Müller.

When injected into the stomach of guinea-pigs treated with tincture of opium according to the method of Koch, about 30 per cent. of the animals die, but the intestinal lesions produced are not identical with those produced by the cholera spirillum. The intestines in such cases are pale and filled with watery material having a strong putrefactive odor. This fluid teems with the spirilla.

It seems unlikely, from the evidence thus far collected, that the Finkler and Prior spirillum is pathogenic for the human species. As Fränkel points out, it is probably a frequent and harmless inhabitant of the human intestine.

The Spirillum of Denecke (Vibrio Tyrogenum).

Another organism with a partial resemblance to the cholera spirillum was found by Denecke * in old cheese.

Morphology.—Its form is similar to that of the cholera spirillum, the shorter individuals being of equal diameter throughout. The spiral forms are longer than those of the

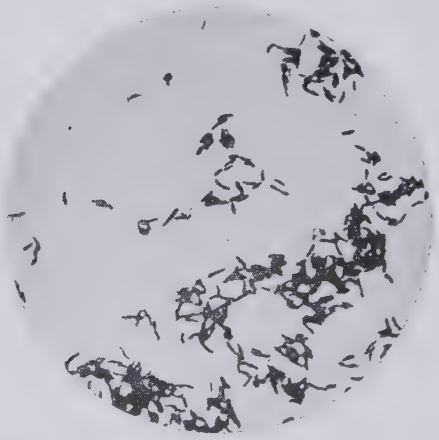


Fig. 137.—Spirillum of Denecke, from an agar-agar culture. × 1000 (Itzerott and Niemann).

Finkler and Prior spirillum, and are more tightly coiled than those of the cholera spirillum.

Like its related species, this micro-organism is actively motile and possesses a terminal flagellum.

Cultivation.—It grows at the room temperature, as well as at 37° C., in this respect, as in its reaction to stains, much resembling the other two.

Colonies.—Upon gelatin plates the growth of the colonies is much more rapid than that of the cholera spirillum, though slower than that of the Finkler and Prior spirillum. The colonies appear as small whitish, round points, which

* "Deutsche med. Wochenschrift," 1885.

soon reach the surface of the gelatin and commence liquefaction. By the second day each is about the size of a pin's head, has a yellow color, and occupies the bottom of a conical depression. The appearance is much like that of colonies of the cholera spirillum.

The microscope shows the colonies to be of irregular shape and coarsely granular, pale yellow at the edges, gradually becoming intense toward the center, and at first circumscribed, but later surrounded by clear zones, resulting from the liquefaction of the gelatin. These, according to

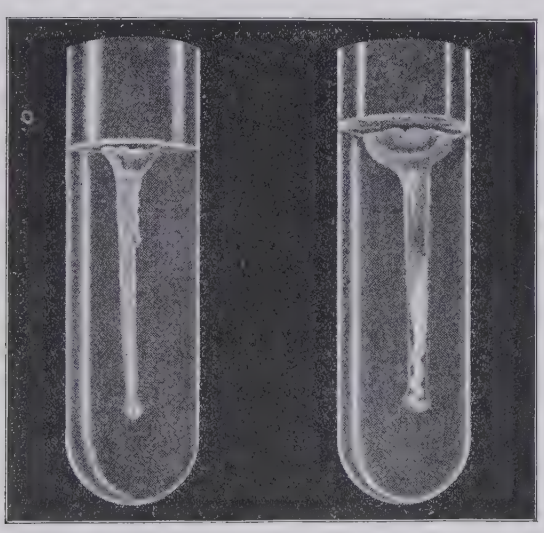


Fig. 138.—Spirillum of Denecke; gelatin puncture cultures aged forty-eight and sixty hours (Shakespeare).

the illumination, appear pale or dark. The colonies differ in appearance from those of cholera in the prompt liquefaction of the gelatin, their rapid growth, yellow color, irregular form, and distinct lines of circumscription.

Gelatin Punctures.—In gelatin punctures the growth takes place all along the track of the wire, and forms a cloudy liquid which precipitates at the apex in the form of a coiled mass. Upon the surface a delicate, imperfect, yellowish scum forms. Liquefaction of the entire gelatin generally requires about two weeks.

Agar-agar.—Upon agar-agar this spirillum forms a thin yellowish layer which spreads quickly over most of the surface.

Bouillon.—In bouillon the growth of the organism is characterized by a diffuse turbidity. No gas-formation occurs in sugar-containing media.

Potatoes.—The culture upon potato is luxuriant if grown in the incubating oven. It appears as a distinct yellowish, moist film, and when examined microscopically is seen to contain beautiful long spirals.

Metabolic Products.—The organism produces no indol.

Pathogenesis.—The spirillum of Denecke is mentioned only because of its morphologic resemblance to the cholera spirillum. It is not associated with any human disease. Experiments, however, have shown that when the spirilla are introduced into guinea-pigs whose gastric contents are alkalinized and whose peristalsis is paralyzed with opium, about 20 per cent. of the animals die.

The Spirillum of Gamaléia* (Vibrio Metschnikovi).

Resembling the cholera spirillum in morphology and vegetation, and possibly, as has been suggested, a descendant of the same original stock, is a spirillum which Gamaléia cultivated from the intestines of chickens affected with a disease similar to chicken-cholera.

Morphology.—This spirillum is a trifle shorter and thicker than the cholera spirillum. It is a little more curved, and has similar rounded ends (Fig. 139). It forms long spirals in appropriate media, and is actively motile. Each spirillum is provided with a terminal flagellum. No spores have been demonstrated.

Staining.—The organism stains easily, the ends more deeply than the center. It is not stained by Gram's method.

Cultivation.—It grows well both at the temperature of the room and at that of incubation.

Colonies.—The colonies upon gelatin plates have a marked resemblance to those of the cholera spirillum, yet there is a difference; and as Pfeiffer says, "it is comparatively easy to differentiate between a plate of pure cholera spirillum and a plate of pure Spirillum metschnikovi, yet it

* "Ann. de l'Inst. Pasteur," 1888.

is almost impossible to pick out a few colonies of the latter if mixed upon a plate with the former."

Fränkel regards this organism as a species intermediate between the cholera and the Finkler-Prior spirilla.

The colonies upon gelatin plates appear in about twelve hours as small whitish points, and rapidly develop, so that by the end of the third day large saucer-shaped liquefactions resembling colonies of the Finkler-Prior spirilla occur. The liquefaction of the gelatin is quite rapid, the resulting fluid being turbid. Usually, upon a plate of Vibrio metschnikovi some colonies are present which closely resemble those of

Fig. 139.—Spirillum metschnikovi, from an agar-agar culture. × 1000 (Itzerott and Niemann).

the cholera spirillum, being deeply situated in conical depressions in the gelatin. Under the microscope the contents of the colonies, which appear of a brownish color, are observed to be in rapid motion. The edges of the bacterial mass are fringed with radiating organisms (Fig. 140).

Gelatin Punctures.—In gelatin tubes the growth closely resembles that of the cholera organism, but develops more slowly.

Agar-agar.—Upon the surface of agar-agar a yellowish-brown growth develops along the whole line of inoculation.

Potato.—On potato at the room temperature no growth occurs, but at the temperature of the incubator a luxuriant

yellowish-brown growth takes place. Sometimes the color is quite dark, and chocolate-colored potato cultures are not uncommon.

Bouillon.—In bouillon the growth which occurs at the temperature of the incubator is quite characteristic, and very different from that of the cholera spirillum. The entire medium becomes clouded, of a grayish-white color, and opaque. A folded and wrinkled pellicle forms upon the surface.

Milk.—When grown in litmus milk, the original blue color is changed to pink in a day, and at the end of another

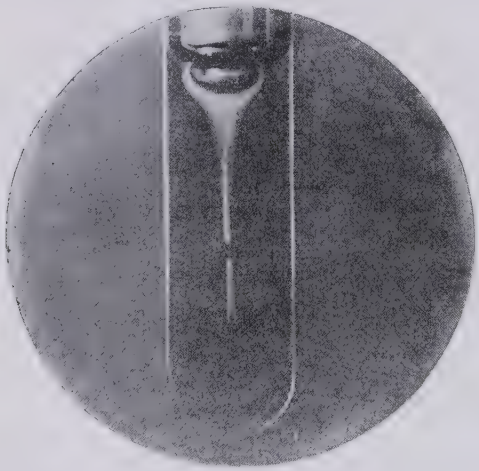


Fig. 140.—Spirillum metschnikovi; puncture culture in gelatin forty-eight hours old (Fränkel and Pfeiffer).

day the color is all destroyed and the milk coagulated. Ultimately the clots of casein sediment in irregular masses, from the clear, colorless whey.

Vital Resistance.—The organism, like the cholera vibrio, is very susceptible to the influence of acids, high temperatures, and drying. The thermal death-point is 50° C., continued for five minutes.

Metabolic Products.—The addition of sulphuric acid to a culture grown in a medium rich in peptone produces the same rose color observed in cholera cultures and shows the presence of indol. When glucose is added to the bouillon,

no fermentation or gas-production results. The organism produces acids and curdling enzymes.

Pathogenesis.—The organism is pathogenic for animals, but not for man. Pfeiffer has shown that chickens and guinea-pigs are highly susceptible, and when inoculated under the skin usually die. The virulent organism is invariably fatal for pigeons. W. Rindfleisch has pointed out that this constant fatality for pigeons is a valuable criterion for the differentiation of this spirillum from that of cholera, as the subcutaneous injection of the most virulent cholera cultures is never fatal to pigeons, the birds only dying when the injections are made into the muscles in such a manner that the muscular tissue is injured and becomes a *locus minoris resistentiæ*. When guinea-pigs are treated by Koch's method of narcotization and cholera infection, the temperature of the animal rises for a short time, then abruptly falls to 33° C. or less. Death follows in from twenty to twenty-four hours. A distinct inflammation of the intestine, with exudate and numerous spirilla, may be found. The spirilla can also be found in the heart's blood and in the organs of such guinea-pigs. When the bacilli are introduced by subcutaneous inoculation, the autopsy shows a bloody edema and a superficial necrosis of the tissues.

The organisms can be found in the blood and all the organs of pigeons and young chickens, in such large numbers that Pfeiffer has called the disease *Vibrionensepticæmia*. In the intestines very few alterations are noticeable, and very few spirilla can be found.

Immunity.—Gamaléia has shown that pigeons and guinea-pigs can be made immune by inoculating them with cultures sterilized for a time at a temperature of 100° C. Mice and rabbits are immune, except to very large doses.

Vibrio Schuylkiliensis (Abbott).

Morphology.—This micro-organism, closely resembling the cholera spirillum, was found by Abbott * in sewage-polluted water from the Schuylkill River at Philadelphia.

Cultivation.—Colonies.—The colonies developed upon gelatin plates very closely resemble those of the Spirillum metschnikovi.

* "Journal of Experimental Medicine," vol. 1, No. 3, July, 1896, p. 419.

Gelatin Punctures.—In gelatin puncture cultures the appearance is exactly like the true cholera spirillum. At times the growth is a little more rapid.

Agar-agar.—The growth on agar is luxuriant, and gives off a pronounced odor of indol.

Blood-serum.—Löffler's blood-serum is apparently not a perfectly adapted medium, but upon it the organisms grow, with resulting liquefaction.

Potato.—Upon potato, at the point of inoculation a thin, glazed, more or less dirty yellow growth, shading to brown and sometimes surrounded by a flat, dry, lusterless zone, is formed.

Milk.—In litmus milk a reddish tinge develops after the milk is kept twenty-four hours at body-temperature. After forty-eight hours this color is increased and the milk coagulates.

Metabolic Products.—In peptone solutions indol is easily detected. No gas is produced in glucose-containing culture media. Acids and coagulating enzymes are formed. The organism is a facultative anaerobe.

Vital Resistance.—The thermal death-point is 50° C. maintained for five minutes.

Pathogenesis.—The organism is pathogenic for pigeons, guinea-pigs, and mice, behaving much like Spirillum metschnikovi. No Pfeiffer's phenomenon was observed with the use of serum from immunized animals.

Immunity.—Immunity could be produced in pigeons, and it was found that the serum was protective against both Vibrio schuylkiliensis and Spirillum metschnikovi, the immunity thus produced being of about ten days' duration.

In a second paper by Abbott and Bergey * it was shown that the vibrios occurred in the water during all four seasons of the year, and in all parts of the river within the city, both at low and at high tide. They were also found in the sewage emptying into the river, and in the water of the Delaware River as frequently as in that of the Schuylkill.

One hundred and ten pure cultures were isolated from the sources mentioned and subjected to routine tests. It was found that few or none of them were identical in all points. There seems to be, therefore, a family of river spirilla, closely related to one another, like the different colon bacilli.

* "Journal of Experimental Medicine," vol. II, No. 5, p. 535.

The opinion expressed is that "the only trustworthy difference between many of these varieties and the true cholera spirillum is the specific reaction with serum from animals immune against cholera, or by Pfeiffer's method of intraperitoneal testing in such animals."

In discussing these spirilla of the Philadelphia waters Bergey * says:

"The most important point with regard to the occurrence of these organisms in the river water around Philadelphia is the fact that similar organisms have been found in the surface waters of the European cities in which there had recently been an epidemic of Asiatic cholera, notably at Hamburg and Altona. . . . The foremost bacteriologists of Europe have been inclined to the opinion that the organisms which they found in the surface waters of the European cities were the remains of the true cholera organism, and that the deviations in the morphologic and biologic characters from those of the cholera organism were brought about by their prolonged existence in water. No such explanation of the occurrence of the organisms in Philadelphia waters can be given."

A number of interesting spirilla, more or less closely resembling that of Asiatic cholera, have been described from time to time. Their variation from the true cholera organism can best be determined by an examination of the following table, though for precise information the student will do well to look up the original descriptions, references to which are given in each case.

* "Jour. Amer. Med. Assoc.," Oct. 23, 1897.

DIFFERENTIAL TABLE FOR SEPARATING ORGANISMS RESEMBLING THE CHOLERA SPIRILLUM.

	Found in Intestinal Diseases.	Found in Water.	Stain by Gram's Method.	Comma Shape.	Thick Spirals.	Slender Spirals.	Spores.	Active Motility.	Terminal Flagella.	Bouillon. Scum and Slight Turbidity.	Bouillon. Scum and Marked Turbidity.	Gelatin. Very Slow Liquefaction.	Gelatin. Slow Liquefaction.	Gelatin. Rapid Liquefaction.	Agar-Agar. Yellowish.	Agar-Agar. Grayish.	Potato. Yellowish.	Potato. Brown.	Potato. Colorless.	Potato. No Growth.	Nitrites.	Indol.	Liquefaction. Gelatin.	Liquefaction. Casein.	Liquefaction. Blood-serum.	Fermentation.	Milk. Alkalinized.	Milk. Acidified.	Milk. Coagulated.	Phosphorescent.	Pathogenic. Pigeons.	Pathogenic. Guinea-pigs.	Pathogenic. Rabbits.	Chromogenic.	Fluorescent.
Intestinal Group.																																		
Spirillum choleræ asiaticæ (Koch*)	+	+	o	+	+	o	o	+	+	+	o	o	+	o	o	+	o	o	+	o	+	+	o	+	o	o	+	o	o	+	o	o	o	o
Spirillum choleræ nostras (Vibrio proteus) (Finkler and Prior†)	+	o	o	+	+	o	o	+	+	+	o	+	o	+	o	+	+	o	+	o	+	+			+	o				+	+		o	o
Spirillum tyrogenum (Denecke‡)	+	o	o	+	+	o	o	+	+	o	+	+	o	+	o	+	+	o	+	o	+	+			+	o				+	+		o	o
Spirillum metschnikovi (Gamaléia§)	+	o	o	+	+	o	o	+	+	+	o	+	o	+	o	+	+	o	+	o	+	+			+	o				+	+		o	o
Water Group.																																		
Spirillum dunbarensis (Dunbar‖)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	o	+	+	o	o	o	+	+			+	o	o			o	+			
Spirillum danubicus (Heider**)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	+	o	+	o	o	o	+	+			+	o		+		o	+		o	o
Spirillum I (Wernicke††)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	o	+	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum II (Wernicke‡‡)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	o	+	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum liquefaciens (Bonhoff§§)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	o	+	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum weibeli (Weibel‖‖)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	+	o	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum milleri (Miller***)	o	o	o	+	+	o	o	+	+	+	o	o	o	o	+	o	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum terrigenus (Günther†††)	o	o	o	+	+	o	o	+	+	+	o	o	o	o	+	o	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum berolinensis (Neisser‡‡‡)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	+	o	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum aquatilis (Günther§§§)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	+	o	+	o	o	o	+	+			+	o				o	+		o	o
Spirillum schuylkiliensis (Abbott and Bergey‖‖‖)	o	+	o	+	+	o	o	+	+	+	o	o	o	o	+	o	+	o	o	o	+	+			+	o				o	+		o	o

* "Berliner klin. Wochenschrift," 1884, Nos. 31 and 32.
§ "Ann. de l'Inst. Pasteur," t. II, 1888, p. 482.
†† "Archiv für Hygiene," XXI, 1894, p. 172.
‖‖ "Centralbl. f. Bakt.," etc., Bd. II, 1887, p. 469.
‡‡‡ "Hygienische Rundschau," 1893.
† "Deutsche med. Wochenschrift," 1884, p. 632.
‖ "Deutsche med. Wochenschrift," 1893, p. 799.
‡‡ "Archiv für Hygiene," XXI, 1894, p. 179.
*** "Deutsche med. Wochenschrift," 1885, p. 138.
‡ "Deutsche med. Wochenschrift," 1885.
** "Centralbl. f. Bakt.," etc., XIV, p. 341.
§§ "Archiv für Hygiene," XIX, 1893, p. 248.
††† "Hygienische Rundschau," IV, 1894, p. 721.
§§§ "Deutsche med. Wochenschrift," 1892, p. 1124.
‖‖‖ "Journal of Experimental Medicine," July, 1896, p. 419, vol. I, No. 3.

(C) THE BACTEREMIAS.

CHAPTER I.

ANTHRAX.

Bacillus Anthracis.

General Characteristics.—A non-motile, non-flagellated, sporogenous, liquefying, non-chromogenic, pathogenic, aerobic bacillus staining by the ordinary methods and by Gram's method.

The disease of cattle known as anthrax, "splenic fever," "*Milzbrand*," and "*charbon*," of infrequent occurrence in this country and England, is a dreaded and common malady in France, Germany, Hungary, Russia, Persia, and the East Indian countries. In Siberia the disease is so common and malignant as to deserve its popular name "Siberian pest." Certain districts, as the Tyrol and Auvergne, in which it seems to be endemic, serve as foci from which the disease spreads in summer, afflicting many animals, and ceasing its depredations only with the advent of winter. It is not rare in the United States, where it seems to be chiefly a disease of the summer season.

The animals most frequently affected are cows and sheep. Among laboratory animals white mice, house-mice, guinea-pigs, and rabbits are highly susceptible; dogs, cats, most birds, and amphibians are immune. White rats are infected with difficulty. Man is slightly susceptible, the disease in the human species usually being a local affection,—"malignant carbuncle,"—commonly succeeded by a general fatal infection.

Anthrax was one of the first infectious diseases proved to depend upon a specific micro-organism. As early as 1849 Pollender * discovered small rod-shaped bodies in the blood of animals suffering from anthrax, but the exact relation which they bore to the disease was not pointed out until 1863, when Davaine,† by a series of interesting experiments,

* "Vierteljahrsschr. für ger. Med.," Bd. VIII, 1855

† "Compte-rendu," 57, 59–61, 77.

proved their etiologic significance to most unbiased minds. The final confirmation of Davaine's conclusions and actual proof of the matter rested with Pasteur and Koch, who, observing that the bacilli bore spores, cultivated them successfully outside the body, and produced the disease by the inoculation of pure cultures.

Morphology.—The anthrax bacillus (Fig. 141) is a large rod-shaped organism, of rectangular form, with slightly rounded corners. It measures 5–20 μ in length and from 1 μ to 1.25 μ in breadth. It has a pronounced tendency

Fig. 141.—Bacillus anthracis; colony three days old upon a gelatin plate; adhesive preparation. × 1000 (Fränkel and Pfeiffer).

to form long threads, in which, however, the individuals can usually be made out, the lines of junction of the component bacilli giving the thread somewhat the appearance of a bamboo rod.

Sporulation.—The formation of endospores is prolific: each spore has a distinct oval shape, is transparent, situated at the center of the bacillus in which it occurs. It does not alter the contour of the bacillus. The spores are formed only in the presence of oxygen upon the surfaces of the culture media. When a spore is placed under conditions favorable to its development, it increases in length and ruptures at the end, from which the new bacillus escapes.

The spores of the anthrax bacillus (Fig. 142), being large and readily obtainable, form excellent subjects for the study of spore-formation and germination, for the study of the action of germicides and antiseptics, and for staining.

Motility.—The bacilli are not motile and have no flagella.

Staining.—They stain well with ordinary solutions of the anilin dyes, and can be beautifully demonstrated in the tissues by Gram's method and by Weigert's modification of it. Picrocarmin, followed by Gram's stain, gives a beau-

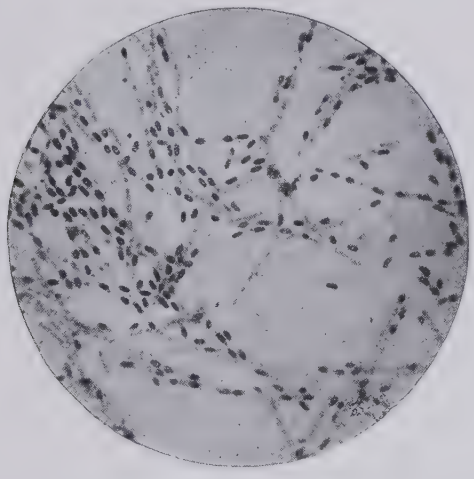


Fig. 142.—Bacillus anthracis, stained to show the spores. × 1000 (Fränkel and Pfeiffer).

tiful, clear picture. The spores can be stained with carbol-fuchsin, the bacilli decolorized with a very weak acid and then counterstained with a watery solution of methyl blue.

Isolation.—The bacillus of anthrax is one of the easiest organisms to secure in pure culture from the tissues and excreta of diseased animals. Its luxurious vegetation, the typical appearance of its colonies, and its infectivity for the laboratory animals combine to make possible its isolation either by direct cultivation from the tissues or by the plate method, or by inoculation into animals and recovery from their blood.

Cultivation.—Colonies.—Upon the surface of a gelatin plate the bacillus forms beautiful and highly characteristic colonies (Fig. 143). To the naked eye they appear first as minute round, grayish-white dots upon the surface. They early begin liquefaction of the gelatin, which progresses rapidly as they increase in size. Under the microscope the smallest colonies are egg-shaped, slightly brown and granular. They do not attain their full development except upon the surface of the medium, where they spread out into flat, irregular, transparent tufts like curled wool. From a tangled center large numbers of curls, made up of parallel

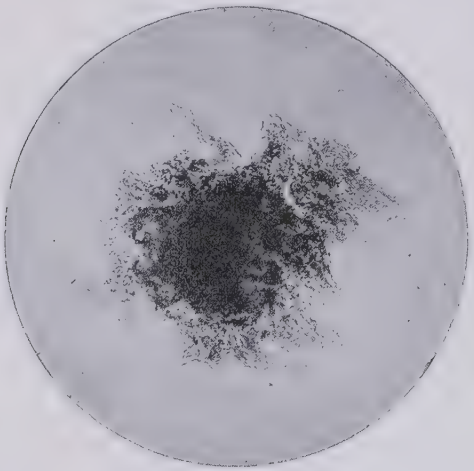


Fig. 143.—Bacillus anthracis; colony upon a gelatin plate. × 100 (Fränkel and Pfeiffer).

threads of bacilli, extend upon the gelatin. As soon as the colony attains to any considerable size liquefaction becomes rapid. Beautiful adhesion preparations can be made if a perfectly clean cover-glass be passed once through a flame and laid carefully upon the gelatin, the colonies being picked up entire as the glass is carefully removed. Such a specimen can be dried, fixed, and stained in the same manner as an ordinary cover-glass preparation.

Gelatin Punctures.—In gelatin puncture cultures the growth is even more characteristic than are the colonies. The bacilli begin to grow along the entire track of the wire, but develop most luxuriantly at the surface, where oxygen

is plentiful and where a distinct shaggy pellicle is formed. From the deeper growth, fine filaments extend from the puncture into the surrounding gelatin, with a beautiful arborescent effect (Fig. 144).

Liquefaction progresses from above downward until ultimately the entire gelatin is fluid and the growth sediments.

Agar-agar. — Upon agar-agar characteristic appearances are few. The growth takes place along the line of inoculation, forming a grayish-white, translucent, slightly wrinkled layer with irregular edges, from which curls of bacillary threads extend upon the medium. When the culture is old, the agar-agar usually becomes brown in color. Spore-formation is luxuriant.

Bouillon.—In bouillon the anthrax bacillus, because of its marked affinity for oxygen, grows chiefly upon the surface, where a thick felt-like pellicle forms. From this, fuzzy extensions descend into the clear bouillon below. After a few days some wooly aggregations can be seen in the bottom of the tube. In the course of time the growth ceases and the surface pellicle sinks. If, by shaking, it is caused to sink prematurely, a new, similar surface growth takes its place. Spore-formation is rapid at the surface.

Potato.—Upon the potato the growth is white, creamy, and rather dry. Sporulation is marked.

Blood-serum.—Blood-serum cultures lack characteristic peculiarities; the culture medium is slowly liquefied.

Fig. 144.—Bacillus anthracis; gelatin stab culture, showing characteristic growth with commencing liquefaction and cupping (from evaporation) at the surface of the medium (Curtis).

Milk.—The anthrax bacillus grows well in milk, which it coagulates. Later the coagulum is peptonized and dissolved, leaving a clear whey. The reaction is not altered.

Thermic Sensitivity.—The bacillus grows between the extremes of 20° and 45° C., best at 37° C. The exposure of the organism to the temperature of 42–43° C. slowly diminishes its virulence.

When dried upon threads, the spores retain their vitality for years, and are highly resistant to heat and disinfectants. The spores of anthrax are killed by five minutes' exposure to 100° C. It is said by some that spores sub-

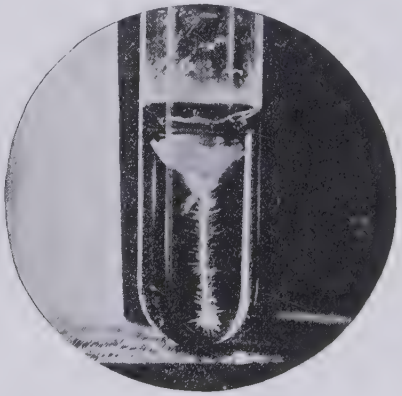


Fig. 145.—Bacillus anthracis; gelatin puncture culture seven days old (Günther).

jected to 5 per cent. carbolic acid can subsequently germinate when introduced into susceptible animals, their resistance to this strength carbolic solution being so great that they are not destroyed by it under twenty-four hours. They are killed in a short time by exposure to 1 : 1000 bichlorid of mercury solution.

Metabolic Products.—The anthrax bacillus produces a curdling ferment. It produces no important change of reaction in the medium in which it grows, and generates no indol. Its proteolytic enzyme is active, digesting both casein and fibrin.

It is doubtful whether the anthrax bacillus produces any important toxic substance. Hoffa * isolated a basic sub-

* "Ueber die Natur. des Milzbrandgifts," Wiesbaden, 1886.

stance from anthrax cultures and called it *anthracin;* Hankin and Wesbrook,* an albumose fatal in large doses and immunizing in small ones. Brieger and Fränkel † isolated a toxalbumin from the tissues of animals dead of anthrax. Martin ‡ separated protalbumose, deuteroalbumose, peptone, an alkaloid, leucin, and tyrosin. The albumoses were not very poisonous, but the alkaloid was capable of producing death after the development of somnolence. The animals were edematous. Marmier § isolated a toxin of non-albuminous nature and immunizing power. Conradi ‖ in an elaborate research failed to find that the anthrax bacillus produces any soluble extracellular or intracellular poison capable of affecting susceptible animals, and concludes that it is highly improbable that the anthrax bacillus produces any toxic substance at all.

Pathogenesis.—Avenues of Infection.—Infection usually takes place through the *respiratory tract,* through the *alimentary canal,* or through wounds. It may take place through the placenta.

When the bacilli are taken into the stomach they are probably destroyed by the acid gastric juice. The spores, however, are able to endure the acid gastric juice, and pass into the intestine, where the suitable alkalinity enables them to develop into bacilli, surround the villi with

Fig. 146.—Bacillus anthracis; glycerin agar-agar culture (Curtis).

* "Ann. de l'Inst. Pasteur," 1892, No. 9.

† "Ueber Ptomaine," Berlin, 1885–1886.

‡ "Proceedings of the Royal Society," May 22, 1890.

§ "Ann. de l'Inst. Pasteur," 1895, p. 533.

‖ "Zeitschrift für Hygiene," June 14, 1899.

thick networks of bacillary threads, separate the covering epithelial cells, enter the lymphatics, and then the blood, from which a general infection occurs.

The bacillus frequently enters the body through wounds, cuts, scratches, and perhaps occasionally fly-bites. Under these conditions the organisms at once find themselves in the lymphatics or capillaries, and may cause immediate general infection. In human beings a "malignant pustule" is apt to follow local infection, and may recover or ultimately cause death by general infection. Those whose occupations bring them in contact with the skins and hair from animals dead of anthrax are liable to the infection.

Anthrax in cattle probably results from the inhalation or ingestion of the spores of the bacilli from the pasture. From the work of Nuttall * it is pretty clear that flies play little part in the transmission of the disease. Interesting discussions arose concerning the infection of the pastures. It was argued that, the bacilli being inclosed in the tissues of the diseased animals, infection of the pasture must depend upon the distribution of the germs from buried cadavers, either through the activity of earthworms, which ate of the earth surrounding the corpse and deposited the spores in their excrement (Pasteur), or to currents of moisture in the soil. Koch seems, however, to have demonstrated the fallacy of both theories by showing that the conditions under which the bacilli find themselves in buried cadavers are opposed to fructification or sporulation, and that in all probability the bacteria suffer the same fate as the cells of the buried animals, and disintegrate, especially if the animal be buried at a depth of two or three meters.

Fränkel points out particularly that no infection of the soil by the dead animal could be worse than the pollution of its surface by the bloody stools and urine, rich in bacilli, discharged upon it by the animal before death, and that it is the live, and not the dead, animals that are to be blamed for the infection.

Lesions.—The disease as seen in the laboratory is accompanied by few marked lesions. The ordinary experimental inoculation is made by cutting away a little of the hair from the abdomen of a guinea-pig or rabbit, or at the root of a mouse's tail, making a little subcutaneous pocket by a snip with sterile scissors, and introducing the

* "Johns Hopkins Hospital Reports," 1899.

spores or bacilli with a heavy platinum wire, the end of which is flattened, pointed, and perforated. An animal inoculated in this way dies, according to the species, in from twenty-four hours to three days, suffering from weakness, fever, loss of appetite, and a bloody discharge from nose and bowels. There is much subcutaneous gelatinous edema near the inoculation wound. The abdominal viscera are injected and congested. The spleen is enlarged, dark in color, and of mushy consistence. The liver is also somewhat enlarged. The lungs are usually slightly congested.

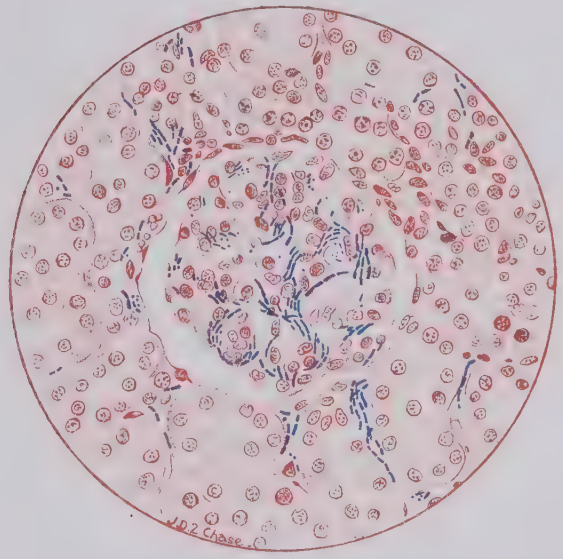


Fig. 147.—Anthrax bacilli in glomeruli of kidney.

When organs which present no appreciable changes to the naked eye are subjected to a microscopic examination, the appropriate staining methods show the capillary and lymphatic systems to be almost universally occupied by bacilli, which extend throughout their meshworks in long threads. Most beautiful bacillary threads can be found in the glomeruli of the kidney and in the minute capillaries of the intestinal villi. In the larger vessels, where the blood-stream is rapid, no opportunity is afforded for the formation of the threads, and the bacteria are relatively

few, so that the burden of bacillary obstruction is borne by the minute vessels. The condition is thus a pure bacteremia.

Death from anthrax seems to depend essentially upon the obstruction of the circulation by the multitudes of bacilli in the capillaries, upon the appropriation of the oxygen destined to support the tissues, by the bacilli, leaving the tissues to be poisoned by the carbon dioxid, rather than upon intoxication by metabolic products of bacillary growth.

Vaccination.—Pasteur * early realized the importance of some practical measure for the protective vaccination of cattle against the disease, and devoted himself to investigating the problem. He found that the inoculation of attenuated bacilli into cows and sheep, and their subsequent reinoculation with mildly virulent bacilli, afforded them immunity against highly virulent organisms. Löffler, Koch, and Gaffky, however, found that these immunized animals were not absolutely protected against intestinal anthrax.

The means of diminishing the virulence of the anthrax bacillus are numerous. Toussaint † first produced immunity in animals by injecting them with sterile cultures of the bacillus, and found that the addition of 1 per cent. of carbolic acid to blood of animals dead of anthrax destroyed the virulence of the bacilli; Chamberland ‡ and Roux found the virulence destroyed when 0.1–0.2 per cent. of bichromate of potassium was added to the culture medium; Chauveau used atmospheric pressure to the extent of six to eight atmospheres and found the virulence diminished; Arloing § found that direct sunlight operated similarly; Lubarsch, that the inoculation of the bacilli into immune animals, such as the frog, and their subsequent recovery from its blood, diminishes the virulence of the bacilli markedly.

The protective inoculations prepared by Pasteur consisted of two cultures of increasing virulence, to be employed one after the other, rendering the vaccinated animals more and more immune. The cultures were prepared, that is, at-

* "Rec. de Méd. vet.," Paris, 1879, p. 193.

† "Compte-rendu Acad. des Sci. de Paris," XCI, 1880, p. 135.

‡ "Ann. de l'Inst. Pasteur," 1894, p. 161.

§ "Compte-rendu de l'Acad. des Sci.," Paris, 1892, CXIV, p. 1521.

tenuated by cultivation at 42° C. for a sufficient length of time, the bacilli forming no spores and gradually losing their virulence at this temperature. The *first vaccine* was kept from fifteen to twenty days at 42° C. It killed mice and guinea-pigs one day old, but was without action on guinea-pigs of adult size. The second vaccine only remained at the temperature of 42° C. for from ten to twelve days and killed mice, guinea-pigs, and occasionally rabbits.

The second vaccine is administered from two to three weeks after the first is given, by hypodermic injection into the tissues of the neck or flank. Of each broth culture about 1 c.c. is administered. The animals frequently become ill.

Pasteur demonstrated the value of his method in 1881 at Pouilly-le-Fort, in a manner so convincing to the entire world that it was immediately put into practice in France. Roger * says that between 1882 and 1894 there were 1,788,677 sheep vaccinated, with a mortality of 0.94 per cent., the previous death-rate having been 10 per cent. There were also 200,962 cattle vaccinated, with a reduction of the death-rate from 5 per cent. to 0.34 per cent.

Chamberland has shown that protective inoculation by Pasteur's method has diminished the death-rate from 10 per cent. for sheep and 5 per cent. for cattle to about 0.94 per cent. for sheep and 0.34 per cent. for cattle, so that the utility of the method is scarcely questionable. The method has been less successful elsewhere than in France, and has sometimes caused the death of the animals to be protected.

Protection against anthrax can be afforded in other ways. Hüppe found that the simultaneous inoculation of bacteria not at all related to anthrax will sometimes cause the animal to recover. Hankin found in the cultures chemic substances, especially an albuminose, that exerted a protective influence. Rettger† prepared "prodigiosus powder" from potato cultures of B. prodigiosus, which when injected into guinea-pigs during experimental anthrax infection prolonged life or induced recovery.

Serum Therapy.—In 1890 Ogata and Jasuhara showed that experiment animals convalescent from anthrax possessed an antitoxic substance in the blood of such strength

* "Les Maladies Infectieuse," II, p. 1489.

† "Jour. of Infectious Diseases," vol. II, No. 4, p. 562, Nov. 25, 1905.

that 1 : 800 parts per body-weight of dog's serum containing the antitoxin would protect a mouse. Similar results have been attained by Marchoux.* Serum therapy in anthrax is, however, of no practical importance either for prophylaxis or treatment, as vaccinating the animals is far cheaper and more satisfactory.

Bacteriologic Diagnosis.—When it is desired to have a bacteriologic diagnosis of anthrax made where no laboratory facilities are at hand, an ear of the dead animal can be inclosed in a bottle or fruit jar and sent to the nearest laboratory where diagnosis can be made. The ear contains so little readily decomposable tissue, that it keeps fairly well, drying rather than rotting. It contains enough blood to enable a bacteriologist to make a successful examination.

Sanitation.—As every animal affected with anthrax is a menace to the community in which it lives,—to the men who handle it as well as the animals who browse beside it,—such animals should be killed as soon as the diagnosis is made, and, together with the hair and skin, be burned, or if this be impracticable, Fränkel recommends that they be buried to a depth of at least 1½–2 meters, so that the sporulation of the bacilli is made impossible. The dejecta should also be carefully disinfected with 5 per cent. carbolic acid solution.

Bacilli Resembling the Anthrax Bacillus.

Bacilli presenting the morphologic and cultural characteristics of the anthrax bacillus, but devoid of any disease-producing power, are occasionally observed. Of these, Bacillus anthracoides of Hüppe and Wood,† Bacillus anthracis similis of McFarland,‡ and Bacillus pseudoanthracis§ have been given special names. What relationship they bear to the anthrax bacillus is uncertain. They may be entirely different organisms, or they may be individuals whose virulence has been completely lost through unfavorable environment.

* "Ann. de l'Inst. Pasteur," November, 1895, t. IX, No. 11, pp. 50–75.

† "Berliner klin. Wochenschrift," 1889. 16.

‡ "Centralbl. f. Bakt.," vol. XXIV, No. 26, p. 556.

§ "Hygienische Rundschau," 1894, No. 8.

CHAPTER II.

TYPHOID FEVER.

Bacillus Typhosus (Eberth-Gaffky).

General Characteristics.—A motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, non-aerogenic, aerobic and optionally anaerobic, pathogenic bacillus, staining by ordinary methods, but not by Gram's method.

Typhoid fever, "typhus abdominalis," enteric fever, "la fievre typhique," is a disease so well known and of such universal distribution, that no introductory remarks concerning it are necessary.

The bacillus of typhoid fever (*Bacillus typhosus*) was discovered in 1880 by Eberth* and Koch,† and was first secured in pure culture from the spleen and lymphatic glands four years later by Gaffky.‡

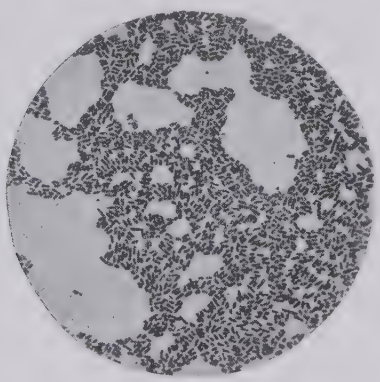


Fig. 148.—Bacillus typhosus, from a twenty-four-hour-old agar-agar culture. × 650 (Heim).

Distribution.—The bacillus is both saprophytic and parasitic. It finds abundant opportunity, in nature, for growth and development, and, enjoying strong resisting powers, can accommodate itself to its environment much better than the majority of pathogenic bacteria, and can

* "Virchow's Archiv," 1881 and 1883.

† "Mittheilungen aus dem kaiserl. Gesundheitsamt," 1, 45.

‡ *Ibid.*, 2.

be found in water, air, soiled clothing, dust, sewage, milk, etc., contaminated directly or indirectly with the intestinal discharges of diseased persons.

The bacillus is also occasionally present upon green vegetables sprinkled with polluted water, and epidemics are reported in which the infection was traced to oysters infected through sewage. Newsholme * found that in 56 cases of typhoid fever about one-third were attributable to eating raw shell-fish from sewage-polluted beds. The bacillus occasionally enters milk in water used to dilute it or to wash the cans.

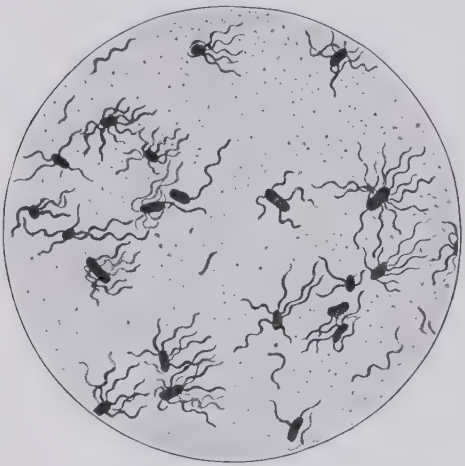


Fig. 149.—Bacillus typhi.

Morphology.—The organism is a short, stout bacillus, about 1–3 μ (2–4 μ—Chantemesse, Widal) in length and 0.5–0.8 μ broad (Sternberg). The ends are rounded, and it is exceptional for the bacilli to be united in chains. The size and morphology vary with the nature of the culture medium and the age of the culture. Thoinot and Masselin,† in describing these morphologic variations, point out that when grown in bouillon the typhoid bacillus is very slender; in milk it is stouter; upon agar-agar and potato it is thick

* "Brit. Med. Jour.," Jan., 1895.

† "Précis de Microbie," Paris, 1893.

and short; and in old gelatin cultures it forms long filaments.

Flagella.—The organisms are actively motile and are provided with numerous flagella, which arise from all parts of the bacillus (peritricha), and are 10–20 in number. They stain well by Löffler's method. The movements of the short bacilli are oscillating; those of the longer bacilli, serpentine and undulating.

Staining.—The organism stains quite well by the ordinary methods, but loses the color when stained by Gram's method. The bacillus gives up its color in the presence of almost any solvent, so that it is particularly difficult to stain in tissue.

When sections of tissue containing the typhoid bacilli are to be stained, the best method is to allow them to remain in Löffler's alkaline methylene-blue for from fifteen minutes to twenty-four hours, then wash in water, dehydrate rapidly in alcohol, clear up in xylol, and mount in Canada balsam. Ziehl's method also gives good results: The sections are stained for fifteen minutes in a solution of distilled water 100, fuchsin 1, and phenol 5. After staining they are washed in distilled water containing 1 per cent. of acetic acid, dehydrated in alcohol, cleared, and mounted. In such preparations the bacilli are always found in scattered groups, which are easily discovered, under a low power of the microscope, as reddish specks, and readily resolved into bacilli with the oil-immersion lens.

In bacilli stained with the alkaline methylene-blue solution, dark-colored dots (Babes-Ernst or metachromatic granules) may sometimes be observed near the ends of the rods.

The typhoid bacillus produces no endospores.

Isolation.—The bacillus is best secured in pure culture, either from an enlarged lymphatic gland or from the splenic pulp of a case of typhoid. To secure it in this way the autopsy should be made as soon after death as possible, lest the colon bacillus invade the tissues.

No special precautions need be taken, as the bacilli are usually present in pure culture. Care must be taken, however, to plant the culture in bouillon containing glucose, and in milk in order that the absence of fermentation and coagulation may make sure that no colon bacilli are present.

As the isolation of a pure culture of the typhoid bacillus from the spleen is sometimes difficult because the groups of bacilli are scattered throughout the organ, E. Fränkel recommends that as soon as the organ is removed from the body it be wrapped in cloths wet with a solution of bichlorid of mercury and kept for three days in a warm room, in order that a considerable and massive development of the bacilli may take place.

Cultures of the typhoid bacillus may also be obtained from the blood, and with much more difficulty from the alvine discharges of typhoid patients during the second and third weeks of the disease.

Numerous methods for facilitating the isolation of the bacillus have been suggested in the hope that they could be practically applied to the diagnosis of the disease. Thus, as numerous saprophytic bacteria are always present in the feces, the resistance of the typhoid bacillus to carbolic acid has been made use of in obtaining the pure culture. To each of several tubes of melted gelatin 0.05 per cent. of carbolic acid is added. This addition is most easily calculated by supposing the average quantity of gelatin contained in a tube to be 10 c.c., when the addition of 0.1 c.c. of a 5 per cent. solution of carbolic acid gives nearly the desired quantity. A minute portion of feces is broken up with a platinum loop and stirred in the tube of melted carbolized gelatin; a drop from this dilution is transferred to the second tube, and a drop from it to a third, and then the contents of each tube are poured upon a sterile plate, into a sterile Petri dish, or rolled, according to Esmarch's suggestion, upon its own walls. The carbolic acid prevents the majority of saprophytes from developing without inhibiting that of the typhoid bacillus and, unfortunately, Bacillus coli communis.

Cultivation.—Colonies.—The deep colonies upon gelatin plates appear under the microscope of a brownish-yellow color and spindle shape, and are sharply circumscribed. When superficial, however, they become larger and form a thin, bluish, iridescent layer with notched edges. The superficial colonies are often described as resembling grape-vine leaves in shape. The center of the superficial colonies is the only portion which shows the yellowish-brown color. The gelatin is not liquefied.

Differentiation.—Unfortunately, the appearances of the

colonies of the typhoid bacillus and the colon bacillus are so similar as to make it impossible to recognize a single colony of either with certainty, and until some satisfactory method for their differentiation is found, the only solution of the problem will be to transfer a large number of colonies to some culture medium, in which specific differences can be recognized, and study the resulting growths.

Special media devised for the purpose of developing the specific differences, such as rapidity of growth, acid-production, etc., are numerous. Thus, Elsner* has suggested the employment of a special medium made by allowing 1

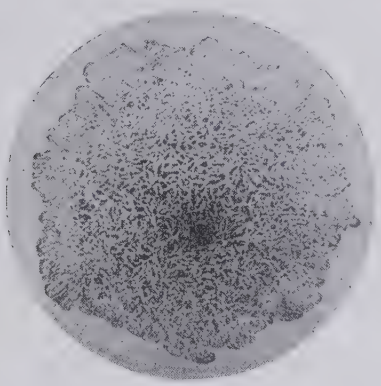


Fig. 150.—Bacillus typhi abdominalis; superficial colony two days old, as seen upon the surface of a gelatin plate. × 20 (Heim).

kilogram of grated potatoes (the small red German potatoes are best) to macerate over night in 1 liter of water. The juice is carefully pressed out, and filtered cold to get rid of as much starch as possible. The filtrate is boiled and again filtered. The next step is a neutralization, for which Elsner used litmus as an indicator, and added 2.5–3 c.c. of a $\frac{1}{10}$ normal sodium hydrate solution to each 10 c.c. of the juice. Abbott prefers to use phenolphthalein as an indicator. The final reaction should be slightly acid. Ten per cent. of gelatin (no peptone or sodium chlorid) is dissolved in the solution, which is boiled, and must then be again neutralized to the same point as before. After filtra-

* "Zeitschrift für Hygiene," XXII, Heft 1, 1895; Dec. 6, 1896.

tion the medium receives the addition of 1 per cent. of potassium iodid; then it is filled into tubes and sterilized like the ordinary culture media.

When water or feces suspected of containing the typhoid bacillus are mixed in this medium and poured upon plates, no bacteria develop well except the typhoid and colon bacilli.

These, however, differ markedly in appearance, for the colon colonies appear of the usual size in twenty-four hours, at which time the typhoid bacillus, if present, will have produced no colonies discoverable by the microscope.

It is only after forty-eight hours—long after the colon colonies have become conspicuous—that little colonies of the typhoid bacillus appear as finely granular, small, round, shining, dew-like points, in marked contrast to their large, coarsely granular predecessors. Unfortunately, many of the small colonies that develop in Elsner's medium subsequently prove to be those of the colon bacillus, and the method is thus rendered unsatisfactory.

Rémy * prefers to make an artificial medium approximating a potato in composition, but without dextrin or glucose. The composition is as follows:

Distilled water	1000.0 grams
Asparagin	6.0 "
Oxalic acid	0.5 gram
Lactic acid	0.15 "
Citric acid	0.15 "
Disodic phosphate	5.0 grams
Magnesium sulphate	2.5 "
Potassium sulphate	1.25 "
Sodium chlorid	2.0 "

All the salts excepting the magnesium sulphate are powdered in a mortar and introduced into a flask with the distilled water. Thirty grams of Witte's or Grubler's peptone are then added and the mixture heated in the autoclave under pressure for one-quarter hour. As soon as removed, the contents are poured into another flask into which 120–150 grams of gelatin had previously been placed. The flask is shaken to dissolve the gelatin, and the contents then made slightly alkaline with soda solution. The mixture is again heated in the autoclave at 110° C. for one-quarter hour, then acidified with a one-half normal solution

* "Ann. de l'Inst. Pasteur," Aug., 1900.

of sulphuric acid, so that 10 c.c. have an acidity neutralized by 0.2 c.c. of one-half normal soda solution. This acidity is equal to 0.5 c.c. sulphuric acid per liter. After shaking, place the flask in a steam sterilizer for ten minutes, then filter. When filtered, verify the acidity of the medium, correcting if necessary. Finally, add the magnesium sulphate, dissolve, dispense in tubes, and sterilize by the intermittent method.

At the moment of using, put into each tube 1 c.c. of a 35 per cent. solution of lactose and 0.1 c.c. of a 2.5 per cent. solution of carbolic acid.

Upon this medium, the colonies of the typhoid and colon bacilli show marked differences. The colon colonies are yellowish-brown, the typhoid colonies bluish-white and small. Fine bubbles of gas from the fermentation of the lactose often occur about the colon colonies.

By this method Rémy was able to isolate the typhoid bacillus from the stools in 23 cases which he studied. He believes that the constant presence of the typhoid bacillus in the stools of typhoid fever, and its absence from them under all other conditions, is a far more important and valuable method of diagnosis than even the Widal reaction.

Kashida * makes the differential diagnosis by observing the acid-production of Bacillus coli in a medium consisting of bouillon containing 1.5 per cent. of agar, 2 per cent. of milk-sugar, 1 per cent. of urea, and 30 per cent. of tincture of litmus. This is the so-called *litmus-lactose-agar-agar*. The culture medium should be blue. When liquefied, inoculated with the colon bacillus, poured into Petri dishes, and stood for from sixteen to eighteen hours in the incubator, the blue color passes off and the culture medium becomes red. If a glass rod dipped in hydrochloric acid be held over the dish, vapor of ammonium chlorid is given off. The typhoid bacillus produces no acid in this medium, and there is consequently no change in its color. Upon plates with colonies of both bacilli, the typhoid colonies produce no change of color, while the colon colonies at once redden the surrounding medium.

Rothberger † first employed *neutral red* for the differentiation of the typhoid and colon bacilli. When grown in fluid media containing it, the colon bacillus produces a yellowish

* "Centralbl. f. Bakt. u. Parasitenk.," Bd. xxi, Nos. 20 and 21, June 24, 1897.

† "Centralbl. f. Bakt.," 1893, p. 187.

fluorescence, while the typhoid bacillus does not destroy the port wine color. Savage* and Irons† have made use of the color reaction for the routine detection of the colon bacillus in water. The best adaptation of the method is by Stokes,‡ who adds it to the various sugar bouillons in the proportion of 0.1 gram per liter, and uses the medium in the fermentation tube. The colon bacillus always ferments the sugars and produces a typical color reaction.

Hiss§ recommends the use of two special media—one for plates, the other for tube cultures. The first consists of 5 grams of agar-agar, 80 grams of gelatin, 5 grams of Liebig's beef-extract, 5 grams of sodium chlorid, and 10 grams of glucose to the liter. The agar is dissolved in the 1000 c.c. of water, to which have been added the beef-extract and sodium chlorid. When the agar is completely melted, the gelatin is added and thoroughly dissolved by a few minutes' boiling. The medium is then titrated to determine its reaction, phenolphthalein being used as the indicator, and enough HCl or NaOH added to bring it to the desired reaction—*i. e.*, a reaction indicating 1.5 per cent. of normal acid. To the clear medium add one or two eggs, well beaten in 25 c.c. of water; boil for forty-five minutes, and filter through a thin layer of absorbent cotton. Add the glucose after clearing.

This medium is used in tubes, in which the culture is planted by the ordinary puncture. *The typhoid bacillus alone has the power of uniformly clouding this medium* without showing streaks or gas-bubbles.

The second medium is used for *plating.* It contains 10 grams of agar, 25 grams of gelatin, 5 grams of beef-extract, 5 grams of sodium chlorid, and 10 grams of glucose. The method of preparation is the same as for the tube medium, care always being taken to add the gelatin after the agar is thoroughly melted, so as not to alter this ingredient by prolonged exposure to high temperature. The preparation should never contain less than 2 per cent. of normal acid. Of all the organisms upon which Hiss experimented, with this medium *Bacillus typhosus alone displayed the power of producing thread-forming colonies.*

The colonies of the typhoid bacillus when deep in Hiss's

* "Jour. of Hyg.," 1901, I, p. 437.

† *Ibid.*, 1902, II, p. 437.

‡ "Jour. of Infectious Diseases," 1904, I, p. 341.

§ "Journal of Experimental Medicine," Nov., 1897, vol. II, No. 6.

medium appear small, generally spheric, with a rough, irregular outline, and by transmitted light of a vitreous greenish or yellowish-green color. The most characteristic feature consists of well-defined filamentous outgrowths, ranging from a single thread to a complete fringe about the colony. The young colonies are, at times, composed solely of threads. The fringing threads generally grow out nearly at right angles to the periphery of the colony.

The colonies of the colon bacillus appear, on the average, larger than those of the typhoid bacillus; they are spheric or of a whetstone form, and by transmitted light are darker, more opaque, and less refractive than the typhoid colonies. By reflected light they are pale yellow to the unaided eye.

Surface colonies are large, round, irregularly spreading, and are brown or yellowish-brown in color. Hiss claims that by the use of these media the typhoid bacillus can readily be detected in typhoid stools.

Piorkowski* recommends a culture medium composed of urine two days old, to which 0.5 per cent. of peptone and 3.3 per cent. of gelatin have been added. Colonies of the typhoid bacillus appear radiated and filamentous; those of the colon bacillus, round, yellowish, and sharply defined at the edges. The cultures should be kept at 22° C., and the colonies should appear in twenty-four hours.

Adami and Chapin† have suggested what seems to be a promising method for the isolation of typhoid bacilli from water, making use of the agglutination of the bacilli by immune serum. Two quart bottles (Winchester quarts) are carefully sterilized and filled with the suspected water with an addition of 25 c.c. of nutrient broth and incubated for eighteen to twenty-four hours at 37° C. By this time the typhoid bacillus grows abundantly in spite of the small amount of nourishment the water contains. At the end of the incubation, 10 c.c. of the fluid is filled into each of a number of long narrow (7 mm.) test-tubes made by sealing a glass tube one-half meter long at one end. About one inch from the bottom the tube is filed completely round so as to break easily at that point. The different tubes next receive additions of typhoid immune serum sufficient to make the dilutions 1 : 60, 1 : 100, 1 : 150, and 1 : 200. If typhoid bacilli are present, within a quarter of an hour beginning agglutination can be seen, and by the end of two to five hours

* "Berliner klin. Wochenschrift," Feb. 13, 1899.

† "Journal of Medical Research," May, 1904, vol. xi, No. 2, p. 469.

flocculent masses collect at the bottom of the tube, forming a flocculent precipitate. The next procedure should be with the tube showing agglutination with the greatest dilution, as the more concentrated preparations carry down not only the typhoid bacilli, but also closely related organisms. After the sedimentation of the agglutinated bacilli is complete, the tube is broken at the file mark, and the sediment contained in the short tube washed with two or three changes of distilled water, being allowed to settle each time. This removes many of the organisms not agglutinated. A loopful of the washed sediment is transferred to a tube of nutrient broth, and finally from this tube plate cultures are made upon Elsner's or Hiss's media.

A culture medium for isolating the typhoid bacillus from feces is recommended by Drigalski-Conradi* and by Petkowitsch.† It is made as follows:

Horse-meat infusion (3 pounds of horse meat to 2 liters of water)	2 liters
Witte's peptone	20 grams
Nutrose	20 grams
Sodium chloride	10 grams
Agar-agar	60 grams
Litmus solution (Kubel and Tiemann)	260 c.c.
Lactose	30 grams
Crystal-violet solution (0.01 per cent.)	20 c.c.

Before adding the crystal-violet solution render feebly alkaline to litmus (about 0.04 per cent. of pure soda).

Colon colonies upon this medium appear in fourteen to sixteen hours to be red and opaque. Typhoid colonies blue or violet, transparent and drop-like.

Gelatin Punctures.—When transferred to gelatin puncture cultures, the typhoid bacilli develop along the entire track of the wire, with the formation of minute confluent, spheric colonies. A small, thin, whitish layer develops upon the surface near the center. The gelatin is not liquefied, but is sometimes slightly clouded in the neighborhood of the growth.

Agar-agar.—The growth upon the surface of obliquely solidified gelatin, agar-agar, or blood-serum is not luxuriant. It forms a thin, moist, shining, translucent band with smooth edges and a grayish-yellow color.

Potato.—When a potato is inoculated and stood in the incubating oven, the typical growth cannot be detected even at the end of the second day, unless the observer be

*"Zeitschrift f. Hygiene," etc., Bd. XXIX.

†"Centralbl. f. Bakt.," etc., May 28, 1904, Bd. XXXVI, No. 2, p. 304.

skilled and the examination thorough. If, however, the surface of the medium be touched with a platinum wire, it is found that its entire surface is covered with a rather thick, invisible layer of a sticky vegetation which the microscope shows to be made up of bacilli. This is described as the *invisible growth*. Unfortunately, it is not a constant characteristic, for occasionally a typhoid bacillus will show a distinct yellowish or brownish color. The typical growth seems to take place only when the reaction of the potato is acid.

Bouillon.—In bouillon the only change produced by the growth of the bacillus is a diffuse cloudiness.

Milk.—In milk a very slight and slow acidity is produced. The milk is not coagulated.

Vital Resistance.—The organisms grow well at all ordinary temperatures. The thermal death-point is given by Sternberg as 60° C. According to Klemperer and Levy,* the bacilli can remain vital for three months in distilled water, though in ordinary water the commoner and more vigorous saprophytes outgrow them and cause their disappearance in a few days. When buried in the upper layers of the soil, the bacilli retain their vitality for nearly six months. Robertson † found that when planted in soil and occasionally fed by pouring bouillon upon the surface, the typhoid bacillus maintained its vitality for twelve months. He suggests that it may do the same in the soil about leaky drains.

Cold has little effect upon typhoid bacilli, for some can withstand freezing and thawing several times. Observing that epidemics of typhoid fever had never been traced to polluted ice, Sedgwick and Winslow ‡ made some investigations to determine what quantitative reduction might be brought about by freezing, and accordingly experimentally froze a large number of samples of water intentionally infected with large numbers of typhoid bacilli from different sources. It was found that the typhoid bacilli disappeared in proportion to the length of time the water was frozen, and that the reduction averaged 99 per cent. in two weeks. The last two or three germs per thousand appeared very resistant and sometimes remained alive after twelve weeks.

* "Clinical Bacteriology."

† "Brit. Med. Jour.," Jan. 8, 1898.

‡ "Jour. Boston Soc. of Med. Sci.," vol. IV, No. 7, p. 181, March 20, 1900.

They have been found to remain alive upon linen from sixty to seventy-two days, and upon buckskin from eighty to eighty-five days. Sternberg has succeeded in keeping hermetically sealed bouillon cultures alive for more than a year. In the presence of chemic agents the bacillus is also able to retain its vitality, from 0.1 to 0.2 per cent. of carbolic acid added to the culture media being without effect upon its growth. At one time the tolerance to carbolic acid was thought to be characteristic, but it is now known to be shared by other bacteria (colon bacillus). The bacilli are killed in a short time by thorough drying.

Metabolic Products.—The typhoid bacillus does not produce indol. It produces a small amount of acid, as is shown by reddening of litmus milk. It forms no coagulating or proteolytic enzymes.

Toxic Products.—The disproportion of local to constitutional disturbance in typhoid fever and the irritative and necrotic character of its lesions suggest that we have to do with a toxic bacterium. Brieger and Fränkel have indeed separated a toxalbumin which they thought to be the specific poison from bouillon cultures. When injected into guinea-pigs the typhotoxin of Brieger causes salivation, accelerated respiration, diarrhea, and mydriasis, and usually leads to a fatal termination in from twenty-four to forty-eight hours. Klemperer and Levy also point out, as affording clinical proof of the presence of toxin, the occasional fatal cases in which the typical picture of typhoid has been without the characteristic postmortem lesions, the diagnosis being made by the discovery of the bacilli in the spleen.

Pfeiffer and Kolle * found toxic substance in the bodies of the bacilli only. It was not, like the toxins of diphtheria and tetanus, dissolved in the culture medium. This was an obstacle to the immunization experiments of both Pfeiffer and Kolle and Löffler and Abel,† for the only method of immunizing animals was to make massive agar-agar cultures, scrape the bacilli from the surface, and distribute them through an indifferent fluid before injecting them into animals.

If the bacilli grown upon ordinary culture media are

* "Deutsche med. Wochenschrift," Nov. 12, 1896.

† "Centralbl. f. Bakt. u. Parasitenk.," Jan. 23, 1896, Bd. XIX, No. 23, p. 51.

several times washed in distilled water, and then allowed to macerate in normal salt solution, autolysis takes place and a toxin is liberated, showing that the toxin is intracellular. Macfadyen and Rowland* liberated an intracellular toxin from cultures of the typhoid bacilli by freezing them with liquid air and grinding them in an agate mortar. Animals immunized with this poison produced an anti-serum active against it, but useless against infection with typhoid bacilli. Wright, of Netley,† observes that Macfadyen's method of securing this intracellular toxin was unnecessarily cumbersome, as the body juices of animals injected with dead cultures of the bacilli dissolve them at once and thus liberate the same toxic product.

Invasion of the Body.—The typhoid bacillus probably, in the great majority of cases, enters the alimentary tract with infected food and water, but may at times be inhaled (Klemperer and Levy).

Pathogenesis.—It is almost universally believed that infection by the typhoid bacillus takes place through polluted drinking-water, and the epidemiology of the disease seems to point unmistakably in that direction. In favor of it as the almost invariable result of purifying the drinking-water is diminution of the number of cases or disappearance of the disease. Opposed to it are the almost invariable failure of bacteriologists to discover the typhoid bacillus in waters thought to be extremely dangerous, and our knowledge of the rapidity with which the typhoid bacillus becomes extinguished in waters containing other micro-organisms. In elaborate experimental studies of this question Jordan, Russell and Zeit‡ found its longevity to be only three or four days under conditions simulating as nearly as possible the conditions found in nature.

The primary operations of the typhoid bacillus are unknown. The resisting power of the organism permits it to pass uninjured through the acid secretions of the stomach and to enter the intestine, where the chief local disturbances are set up. Whether during an early residence in the intestine its metabolism is accompanied by the formation of a toxic product, irritating to the mucosa, and affording the bacilli means of entrance to the lymph-vessels through

* "Brit. Med. Jour.," 1903.

† "Brit. Med. Jour.," April 4, 1903, 1, p. 786.

‡ "Journal of Infectious Diseases," 1904, 1, p. 641.

diminutive breaches of continuity, is not known. We usually find it well established in the intestinal and mesenteric lymphatics at the time we are able to recognize the disease, though in rare cases it appears able to reach the blood through other than the customary channels and occasion an entirely different pathologic picture.

There is always well-marked blood-infection during the first couple of weeks of the disease, and upon this depends the occurrence of the rose-colored spots.

The bacilli enter the solitary glands and Peyer's patches, and multiply slowly during the incubation period of the

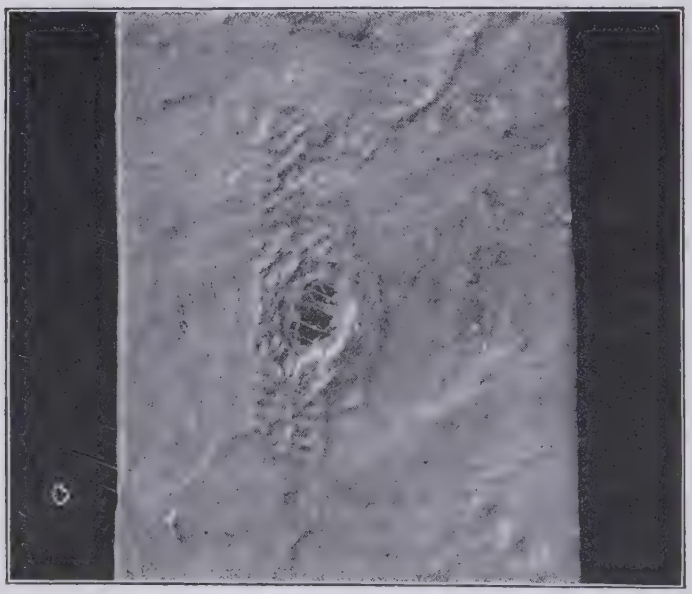


Fig. 151.—Intestinal perforation in typhoid fever. Observe the threads of tissue obstructing the opening. (Museum of the Pennsylvania Hospital.) (Keen, "Surgical Complications and Sequels of Typhoid Fever.")

disease—one to three weeks. The immediate result of their activity in the lymphatic structures is an increase in the number of cells, and ultimately necrosis and sloughing of the Peyer's patches and solitary glands (Fig. 151). From the intestinal lymphatics the bacilli pass, in all probability, to the mesenteric glands, which become enlarged, softened, and sometimes rupture. They also invade the spleen, liver,

and sometimes the kidneys, and other organs where they may be found always aggregated in small clusters in properly stained specimens. The occurrence of the bacilli in the tissues in clumps or clusters may depend upon the presence of agglutinating substances in the blood.

Mallory * found the histologic lesions of typhoid fever to be widespread throughout the body and not limited to the Peyer's patches of the intestine, where they are most evident. His conclusions regarding the pathogenesis of the disease are briefly: "The typhoid bacillus produces a mild diffusible toxin, partly within the intestinal tract, partly within the blood and organs of the body. This toxin produces proliferation of the endothelial cells, which acquire for a certain length of time malignant properties. The new-formed cells are epithelioid in character, have irregular, lightly staining, eccentrically situated nuclei, abundant, sharply defined, acidophilic protoplasm, and are characterized by marked phagocytic properties. These phagocytic cells are produced most abundantly along the line of absorption from the intestinal tract, both in the lymphatic apparatus and in the blood-vessels. They are also produced by distribution of the toxin through the general circulation, in greatest numbers where the circulation is slowest. Finally, they are produced all over the body in the lymphatic spaces and vessels by absorption of the toxin eliminated from the blood-vessels. The swelling of the intestinal lymphoid tissue of the mesenteric lymph-nodes and of the spleen is due almost entirely to the formation of phagocytic cells. The necrosis of the intestinal lymphoid tissue is accidental in nature and is caused through occlusion of the veins and capillaries by fibrinous thrombi, which owe their origin to degeneration of phagocytic cells beneath the lining endothelium of the vessels. Two varieties of focal lesions occur in the liver: one consists of the formation of phagocytic cells in the lymph-spaces and vessels around the portal vessels under the action of the toxin absorbed by the lymphatics; the other is due to obstruction of liver capillaries by phagocytic cells derived in small part from the lining endothelium of the liver capillaries, but chiefly by embolism through the portal circulation of cells originating from the endothelium of the blood-vessels of the intestine and spleen. The liver-cells lying between the occluded capillaries undergo necrosis and disappear. Later the foci of cells degenerate

* "Journal of Experimental Medicine," vol. III, 1898, p. 611.

and fibrin forms between them. Invasion by polymorphonuclear leukocytes is rare."

". . . Histologically the typhoid process is proliferative and stands in close relationship to tuberculosis, but the lesions are diffuse and bear no intimate relation to the typhoid bacillus, while the tubercular process is focal and stands in the closest relation to the tubercle bacillus."

The growth of the bacilli in the kidneys causes albuminuria, and the bacilli can be found in the urine in about 25 per cent. of the cases. Smith * found them in the urine in 3 out of 7 cases which he investigated; Richardson,† in 9 out of 38 cases. They did not occur before the third week, and remained in one case twenty-two days after cessation of the fever. Sometimes they were present in immense numbers, the urine being actually clouded by their presence. Petruschky ‡ found that albuminuria sometimes occurs without the presence of the bacilli; that their presence in the urine is infrequent; that the bacilli never appear in the urine in the early part of the disease, and hence are of little importance for diagnostic purposes. Gwyn § has found as many as 50,000,000 typhoid bacilli per cubic centimeter of urine, and mentions a case of Cushing's in which the *bacilli persisted in the urine for six years after the primary attack of typhoid fever.* Their occurrence, no doubt, depends primarily upon a typhoid bacteremia, by which they are brought to the kidney. After recovery from typhoid fever, their persistence in the urine depends upon continued growth in the bladder and not upon continuous escape from the blood. It is of importance from a sanitary point of view to remember that the urine as well as the feces is infectious.

The bacilli pass from the lymphatics to the general circulation, so that all cases of typhoid fever are true *bacteremias* during part or all of their course.

Ordinarily few bacilli can be found in the circulatory blood, but blood from the roseolæ contains them, and the eruption may be regarded as one of the local irritative manifestations of the bacillus.

Particularly careful work upon this subject has been

* "Brit. Med. Jour.," Feb. 13, 1897.

† "Journal of Experimental Medicine," May, 1898.

‡ "Centralbl. f. Bakt. u. Parasitenk.," May 13, 1898, No. 13, p. 577.

§ "Phila. Med. Jour.," March 3, 1900.

done by Richardson,* who found that by carefully disinfecting the skin, freezing it with chlorid of ethyl, making a crucial incision, and cultivating from the blood thus obtained, he was able to secure the typhoid bacillus in thirteen out of fourteen cases examined. It was, however, necessary to examine a number of spots in each case.

As a means of diagnosis the matter is of some importance, as the occurrence of rose spots may precede the occurrence of the Widal reaction by a number of days.

The pyogenic power of the typhoid bacillus was first pointed out by A. Fränkel, who observed it in a suppuration that occurred four months after convalescence. Löw † found virulent typhoid bacilli in the pus of abscesses occurring from one to six years after convalescence.

Weichselbaum has seen general peritonitis from rupture of the spleen in typhoid fever with escape of the bacilli. Otitis media, ostitis, periostitis, and osteomyelitis are very common results of the lodgment of the bacilli in bony tissue; and Ohlmacher ‡ has found the bacilli in suppurations of the membranes of the brain. The bacilli are also encountered in other local suppurations occurring in or following typhoid fever. Flexner and Harris § have seen a case in which the distribution of the bacilli was sufficiently widespread to constitute a real septicemia, the bacillus being isolated from various organs of the body.

The bacilli commonly escape from the blood into the bile, where they persist for a long time, as in the case studied by Miller, ‖ when they were found in this viscus *seven years* after recovery from typhoid fever, and the case of Humer,** who found them in the gall-bladder of a patient suffering from cholecystitis, *eighteen* years after recovery from an attack of typhoid fever. Cushing †† invariably found the bacilli in the bile in clumps resembling the agglutinations of the Widal reaction. He thinks it probable that these clumps form nuclei upon which bile salts can be precipitated

* "Phila. Med. Jour.," March 3, 1900.

† "Sitz. der k. k. Gesellschaft d. Aerzt. in Wien," "Aerztl. Central-Anz.," 1898, No. 3.

‡ "Jour. Amer. Med. Assoc.," Aug. 28, 1897.

§ "Bull. Johns Hopkins Hospital, Dec., 1897.

‖ *Ibid.*, May, 1898.

** "Bull. of the Johns Hopkins Hospital," Aug. and Sept., 1899.

†† *Ibid.*, IX, No. 86.

and calculus-formation begun. The presence of gall-stones, together with the long-lived infective agents, may at any subsequent time provoke a cholecystitis. Cushing collected six cases of operation for cholecystitis with calculi in which typhoid bacilli were present, and five in which Bacillus coli communis was present in the gall-bladder.

Lower Animals.—Typhoid fever is communicable to animals with difficulty. They are not infected by bacilli contained in fecal matter or by pure cultures mixed with the food, and are not injured by the injection of blood from typhoid patients. Gaffky failed completely to produce any symptoms suggestive of typhoid fever in rabbits, guinea-pigs, white rats, mice, pigeons, chickens, and calves, and found that Java apes could feed daily upon food polluted with typhoid germs for a considerable time, yet without symptoms. Grünbaum* produced typhoid fever in the chimpanzee by inoculation with the Eberth-Gaffky bacillus. This seems to prove its specific nature. The introduction of virulent cultures into the abdominal cavity of animals is followed by peritonitis.

Germano and Maurea† found that mice succumbed in from one to three days after intraperitoneal injection of 1 or 2 c.c. of a twenty-four hour old bouillon culture. Subcutaneous injections in rabbits and dogs caused abscesses.

Lösener found the introduction of 3 mgr. of an agar-agar culture into the abdominal cavity of guinea-pigs to be fatal.

When the gastric contents of animals are rendered alkaline, a large quantity of laudanum injected into the peritoneal cavity, and the bacilli introduced through an esophageal catheter, Klemperer, Levy, and others found that an intestinal condition which very much resembled typhoid as it occurs in man was produced. The virulence of the bacillus can be very greatly increased by rapid passage from guinea-pig to guinea-pig.

Virulent cultures of the typhoid bacillus, when injected subcutaneously into guinea-pigs and some other laboratory animals, produce a fatal septic infection.

In the experiments of Chantemesse and Widal the symp-

* "Brit. Med. Jour.," April 9, 1904.

† "Ziegler's Beiträge," Bd. XII, Heft 3, p. 494.

toms following the injection of virulent culture into guinea-pigs were briefly as follows:

"Very shortly after the inoculation there is a rise of temperature, that continues from one to four hours, and is succeeded by a depression of the temperature, which continues to the fatal issue. Meteorism and great tenderness of the abdomen are observed. At the autopsy a sero-fibrinous or sero-purulent peritonitis is observed—sometimes hemorrhagic. There is also generally a pleurisy, either serous or hemorrhagic. All the abdominal viscera are congested. The intestine is congested—contains an abundant mucous secretion. The Peyer patches are enlarged. The spleen is enlarged, blackish, and often hemorrhagic. In cases which are prolonged the liver is discolored. The kidneys are congested, the adrenals filled with blood.

"In such cases the bacillus can be found upon the inflamed serous membranes, in the inflammatory exudates, in the spleen in large numbers, in the adrenals, the liver, the kidneys, and sometimes in the lungs. The blood is also infected, but to a rather less degree.

"In cases described as chronic, the bacillus disappears completely in from five to twenty-four hours, and produces but one lesion, a small abscess at the point of inoculation.

"Sanarelli has observed that if some of the poisonous products of the colon bacillus or Proteus vulgaris be injected into the abdominal cavity of an animal recovering from a chronic case, it speedily succumbs to typical typhoid fever."

Petruschky * found that mice convalescent from subcutaneous injections of typhoid cultures frequently suffered from a more or less widespread necrosis of the skin at the point of injection.

Prophylaxis.—One of the most important and practical points for the physician to grasp in relation to the subject of typhoid fever is the highly virulent character of the discharges, *both feces and urine.* In every case the greatest care should be taken for their proper disinfection, a rigid attention paid to all the details of cleanliness in the sick-room, and the careful sterilization of all articles which are soiled by the patient. If country practitioners were as careful in this particular as they should be, the disease would be much less frequent in regions remote from the filth and squalor of large cities with their unmanageable slums, and the distribution of the bacilli to villages and towns, by watercourses polluted in their infancy, might be checked.

Antityphoid Serums.—Animals can be immunized to this bacillus, and then, according to Chantemesse and Widal, develop antitoxic blood capable of protecting other

* "Zeitschrift für Hygiene," 1892, Bd. XII, p. 261.

animals. Stern* found in the blood of human convalescents a substance thought to have a protective effect upon infected guinea-pigs. His observation is in accordance with a previous one by Chantemesse and Widal, and has recently been abundantly confirmed.

The immunization of dogs and goats by the introduction of increasing doses of virulent cultures has been achieved by Pfeiffer and Kolle† and by Löffler and Abel.‡ From these animals immune serums were secured.

The typhoid immune serum is specific, but its action requires the presence of additional complementary substance, and by itself it is useless. Indeed, by itself it may do harm by causing the formation of anti-immune bodies.

So far, no serum has been produced that is of any value in therapeutics.

Serum Diagnosis.—The specific action of the artificially prepared serums can be used to differentiate cultures of the colon, paracolon, typhoid, and paratyphoid bacilli, the typhoid bacilli alone exhibiting the specific effect of the typhoid serum.

Richardson§ has found it very convenient to saturate filter paper with typhoid serum, dry it, cut into 0.5 cm. squares, and keep it on hand in the laboratory for the purpose of making this differentiation. To make a test, one of these little squares is dropped in 0.5 c.c. of a twenty-four-hour-old bouillon culture of the suspected bacillus and allowed to stand for five minutes. A drop of the fluid placed upon a slide and covered will then show typical agglutinations if the culture be one of the typhoid fever bacillus. In a second mention of this method‖ he has found its use satisfactory in practice and the paper serviceable after fourteen months' keeping.

Christophers** found that the serum from typhoid patients occasionally caused agglutination of the colon bacillus, but concludes that this does not lessen the specificity of the reaction, as there may be two combined specific actions of the serums. Experiments on rabbits have shown that both typhoid and colon immune serums can be produced, each specific in its agglutinating power upon cultures of its respective organisms.

* "Zeitschrift für Hygiene," 1894, XVI, p. 458.

† "Centralbl. f. Bakt. u. Parasitenk.," Jan. 23, 1896, Bd. XIX, No. 23, p. 51.

‡ *Ibid.*, 1896.

§ "Centralbl. f. Bakt. u. Parasitenk.," 1897, p. 445.

‖ "Journal of Experimental Medicine," May, 1898, p. 353, note.

** "Brit. Med. Jour.," Jan. 8, 1898.

Löffler and Abel also prepared a colon immune serum that exerted a specific action upon the colon bacillus, but was without effect upon the typhoid bacillus.

Widal Reaction of Agglutination.—In 1896 Widal and Grünbaum,* working independently, discovered that when blood-serum from typhoid fever patients is added to cultures of the typhoid bacillus a definite reactive phenomenon occurs identical with that already described by Charrin and Roger and Gruber and Durham (see "Agglutination"). The phenomenon, now familiarly known as the "Widal reaction," consists of complete loss of the motion so characteristic of the typhoid bacillus, and collection of the microorganisms into clusters or groups—agglutination. The bacteria frequently appear shrunken and partly dissolved.

The previous work of Gruber and his associates merits careful study (see chapter upon "Infection"), but so long as the matter was in Gruber's hands it was an interesting observation. Widal developed it into an accurate means of diagnosticating typhoid fever and other diseases.

The reaction usually appears by the seventh day of the disease, sometimes earlier. Johnston and McTaggart † usually found it quite well marked by the fifth day, and sometimes observed it forty-eight hours after the beginning of the fever. Widal and Sicard ‡ state that the reaction occurs on the first day of infection. Not infrequently it fails to develop until later, but most cases react well in the second week, and all show reaction in the third week.

In between 4 and 6 per cent. of cases of clinical typhoid fever the reaction fails to appear even though the case prove fatal. These are probably cases of paratyphoid infection.

In 1899 I § made a large number of blood-examinations to determine the value of the serum test as an aid in diagnosis. The Widal test was made day after day in 230 cases of typhoid fever affecting soldiers of the United States army during the Spanish-American War returned from the camps to be treated in the Medico-Chirurgical Hospital. Of these, 219 reacted positively, or 95.64 per cent. of the total number examined. One of the fatal cases, which was otherwise typical typhoid fever, failed to show a reaction so late as the seventeenth day of the illness; this was excluded, for the obvious reason that had the patient survived for a longer period a positive reaction might have been obtained. So in 10 cases no reaction was present, although the blood was repeatedly tested until the return of the temperature to normal. The percentage of cases, therefore, in which the reaction failed was 4.36 per cent.

Of the 219 cases giving a positive result, 128 showed the reaction prior to the appearance of the rose-spots, or before the eighth day;

* "La Semaine médicale," 1896, p. 295.

† "Amer. Medico-Surgical Bulletin," Jan. 10, 1897, p. 12.

‡ "Ann. de l'Inst. Pasteur," May, 1897.

§ "Phila. Med. Jour.," April 8–15, 1899.

36 showed the reaction during the second week; 45 between the seventeenth and twenty-first days of the disease; 8 not until the twenty-fifth day; and 2 as late as the twenty-eighth day of the illness.*

In addition to the 230 cases considered above, in which the clinical symptoms and course were typical, the presence of the Widal reaction was noted in 10 atypical cases, and lacking in the characteristic features of typhoid fever.

Delepine † found that during the first week the reaction was often slow and not clear, and that to establish an assured diagnosis re-examination was often necessary.

In Osler's wards in the Johns Hopkins Hospital, Block and Gwyn, up to November, 1898, showed that in 151 cases the reaction was present in 144. "In 4 of the negative cases the clinical course was not certain. In only 46 of the last 108 cases was the reaction obtained; in only 26 cases was the reaction present before the seventh day of the disease"; in 4 cases it developed on the twenty-second, twenty-sixth, thirty-fifth, and forty-second days respectively.

In rare cases the reaction may be absent. There are also cases in which the reaction is missed during the primary attack, or until the period of convalescence (Achard, Blumenthal), and, in still others, in which it first makes its appearance in the relapse (Biggs, Park, Stahl, Bell, *et al.*).

Stengel and Kneass ‡ collected and tabulated 2392 cases of typhoid in which the reaction was positive in 2283 and negative in 109; also 1387 non-typhoid cases, of which 22 reacted positively and 1365 negatively. The results in these cases show—

Reactions in typhoid cases	95.5 per cent.
No reactions in non-typhoid cases	98.4 "
Correct results in	96.5 "
Incorrect results in	3.5 "

This table indicates a small proportion of negative results and accentuates the value of the method for diagnosis.

Our present knowledge of the paratyphoid bacillus and its ability to occasion disease resembling clinical typhoid fever enables us to explain these irregularities as probable infections by bacilli other than the true typhoid organism.

The reaction is not permanent. The agglutinating property of the blood usually begins to diminish in the first weeks of convalescence, and marked reduction in its activity is noticed in a few months. Few cases continue to show it longer than one year. Widal and Sicard § found that it may disappear as early as the eighteenth day after convalescence.

In a series of 30 cases that I studied some years ago, the attack of typhoid fever having taken place from one month to twenty years previously, I found the reaction positive in most cases up to the eighth year, doubtful at the ninth year, and absent in all cases after the ninth year. Musser and Swan‖ observed a case in which the reaction remained positive for ten years.

* These results may be misleading, as they might seem to indicate that the blood of these cases was tested every day and the reaction first noted on the day given. In reality the blood was sometimes not examined until the day mentioned.

† Allbutt's "System of Medicine," vol. III, p. 1147.

‡ "Amer. Year-book of Med. and Surg." for 1898.

§ "Compte-rendu de la Soc. de Biol.," Dec. 19, 1896, No. 33.

‖ "Jour. Amer. Med. Assoc.," Aug. 14, 1897.

This occasional persistence of the reaction for years can lead to errors of diagnosis, but is not apt to do so if the previous history of the case can be properly studied.

The agglutinating substance is present in the blood in the various secretions extracted from it. Widal * says it has been found in the blood, urine, the serous fluid from blisters, the pleural, pericardial, and peritoneal fluids, milk, bile, seminal fluid, aqueous humor, tears, pleural exudates, and to a less extent in the spleen, liver, and mesenteric glands. Catrin † found it in the pus of a phlegmonous inflammation occurring during typhoid fever, and Block in the typhoid stools. Thiercelin ‡ found it absent from the spontaneous sweat when present in the blood and milk.

The fact that the typhoid bacilli are scattered throughout the body in small groups suggests that agglutination takes place in the circulating blood of the infected individual. Salimbeni, however, asserts that such agglutinations do not occur.

The relation of the reaction to the germicidal activity of the blood has been carefully studied, with the result of finding that the two phenomena are different and depend upon different causes, though occasionally present simultaneously in the same blood. It is only in fresh blood that bacteriolysis is observed. Johnston and McTaggart § found that "with blood-solutions this phenomenon is frequently witnessed. The clumped bacteria, if watched for an hour or so, may be seen to break up into granules which gradually become indistinct and vanish while under observation, until practically no trace remains of the clumps which shortly before studded the entire field of the microscope. The change is more liable to occur in cultures some days old than in young cultures, and more likely with attenuated than with virulent cultures."

The agglutinative substance is different from the bactericidal substance, and the agglutination of the bacteria is not to be looked upon as the beginning of their destruction. Agglutination takes place as the result of contact with immune serum; bacteriolysis, only when the bacteria, immune body, and proper complementary bodies are all simultaneously present. Many typhoid serums with a high degree of agglutinating power are entirely devoid of bactericidal powers.

Jemmal ‖ found that the phenomenon of agglutination was most marked during the period of most intense infection and when the bactericidal activity was greatest. Widal and Sicard ** also trace a relationship between the two; but they were able to keep typhoid cultures alive for two months in strongly agglutinative serums without destroying their vitality; and indeed, one of the original methods that Widal suggested for studying the reaction required that the typhoid bacillus should *grow* in diluted, but of course agglutinative, serum.

Mosse and Dannie †† observed a case of typhoid during the eighth month of pregnancy. At the time of delivery the blood and mammary secretion of the mother and the blood of the child gave a positive

* "Ann. de l'Inst. Pasteur," May, 1897, No. 5.

† "Gaz. de Méd. de Paris," Oct. 15, 1896.

‡ "Compte-rendu de la Soc. de Biol.," Dec. 19, 1896, No. 33.

§ "Montreal Med. Jour.," March, 1897.

‖ "Centralbl. f. innere Med.," Jan. 23, 1897.

** "Compte-rendu de la Soc. de Biol.," March, 1897, No. 8.

†† Philadelphia Pediatric Society, 1897.

reaction for thirty-three days after birth. J. P. C. Griffith,* Barber,† Pepper and Stengel,‡ and others have seen similar cases. Charrier and Apert § examined an embryo aborted by a mother in the third week of typhoid fever, in which there was a total absence of any agglutinating property in the blood of the fetus, though it was present in the blood of the placenta.

The specific nature of the reaction is now universally accepted. Normal human serum, when concentrated, occasionally exerts a slight agglutinating effect upon the typhoid bacillus, but this seldom occurs except with concentrations exceeding those employed for diagnostic purposes.

The blood of certain animals normally possesses an agglutinating property for the typhoid and other bacilli. Some individuals are peculiar in that their blood acts more strongly in its agglutinating and bacteriolytic action than others.

The blood in a few diseases that may or may not be related to typhoid (paratyphoid) occasionally produces a reactive effect upon the typhoid bacillus. The number of cases in which such errors can occur is very small, and the validity of the test is very slightly influenced by them.

Fritz Köhler ‖ found that the blood in chlorosis occasionally gave the agglutinative phenomenon with the typhoid bacillus, and that in icterus the blood was very apt to do so. In dogs whose common bile-ducts were ligated so that icterus developed, the blood nearly always developed it. The agglutinating substance in icterus is thought to be *taurocholic acid.*

Villiez and Battle ** found a positive reaction in a case of malaria from Madagascar.

The reactive phenomena are very slightly interchangeable for species of bacteria intermediate between the typhoid and colon bacilli. Far from lessening the value of the test, this, as Welch points out, only argues for the close relationship of the species acted upon.

The typical reaction does not occur with allied members of the typhoid group of bacilli. Attempts to make the colon bacillus agglutinate by the application of typhoid serum fail. In suspected typhoid, in which the reaction upon the colon bacillus takes place, it is impossible to eliminate the possibility of combined typhoid and colon bacillus infection, as suggested by Johnston and McTaggart, and the paratyphoid bacillus and paratyphoid fever must not be forgotten.

Widal and Courmont found that all human serums, whether normal or typhoid, have a slight action upon the colon bacillus in dilutions of 1 : 10, whereas normal serum, as a rule, has no effect upon the typhoid bacillus in this dilution. This may indicate that the constant presence of the colon bacillus in the alimentary canal may occasion the presence of some immune substance in presumably normal blood.

The Technic of Widal's Reaction.—Widal †† first suggested that, to make the test, a small quantity of blood be withdrawn in a sterile syringe from the median cephalic vein, and a few drops of it be added to a fresh bouillon culture of the typhoid bacillus in the proportion

* See "Amer. Jour. Med. Sci.," N. S., Jan.–June, 1897, vol. CXIII, p. 621.

† "New York Med. Jour.," April 16, 1898, vol. LXVII, No. 16.

‡ "Year-book of Medicine," 1897.

§ "Compte-rendu de la Soc. de Biol. de Paris," Jan. 1, 1897.

‖ "Centralbl. f. Bakt. u. Parasitenk.," May 22, 1901, XXIX, No. 17, p. 683.

** "La Presse méd.," 1896, No. 84.

†† "La Semaine médicale," 1896, p. 259.

of 1 : 10 or 1 : 15, etc. Twenty-four hours afterward the cloudiness characteristic of the growth of the typhoid bacillus in bouillon had entirely disappeared, because the bacilli, massed together, had all sedimented, and were found as a flocculent sediment at the bottom of the tube. This he called the "rapid method," in contradistinction to a more rarely employed "slow or culture method," in which the serum in the given proportion was added to the sterile bouillon, which was inoculated with the typhoid bacillus and stood in an incubating oven for about fifteen hours, or until the growth could be observed in a flocculent mass at the bottom of the tube. Pugliesi * used serum obtained from blisters for the same purpose.

Both these methods were so annoying to the patient that, had no improvement in the technic been devised, it is probable that the reaction would have attained little importance as a method of diagnosis. Widal himself made the first improvement, and suggested that instead of a syringeful of blood a few drops be secured from the finger-tip. He also recommended observing the reaction through the microscope instead of awaiting the results of the slow sedimentation that succeeds the addition of the blood to a culture, and found that it was unnecessary to use fresh serum, as serum that had been kept for some time produced all the phenomena in a typical manner.

Wyatt Johnston,† independently of Widal, found that successful reactions could be obtained from blood dried upon paper and redissolved in water. The practical use of his observation was made by having all specimens of blood to be tested by the Widal method dried upon paper and sent to the laboratory at Montreal, where they could be studied and reported upon. A ready and certain means of diagnosis of doubtful cases of typhoid fever having been long desired, this method met with ready acceptance. Indeed, the outcome of Johnston's work was the establishment, at the laboratory of the Board of Health of the Province of Quebec, and later at most public laboratories in this country, of a system of free examinations of typhoid bloods, by which the physicians of the larger cities and towns can have their diagnoses confirmed.

The paper upon which the blood is dried is moistened with germ-free water, and a drop of the solution placed upon a cover-glass that has just been passed through a flame. A drop of a bouillon culture, or of a watery suspension of an agar-agar culture of the typhoid bacillus, is mixed with it, the cover placed upon a concave slide to make a "hanging drop" (*q. v.*), and is then examined under a dry lens of moderate power (one-fourth or one-fifth inch). This was Johnston's technic. Wesbrook endeavored to improve it by having the blood sent to the laboratory dried upon carefully weighed pieces of tin-foil, prepared for the purpose in the laboratory. The advantage of this is that the exact weight of the dried blood can be determined, and accurate dilutions made.

Cabot has made use of the medicine dropper: he secures blood from the finger and drops it into a receptacle, afterward adding a given number of drops of bouillon culture from the same medicine dropper.

Robin ‡ has improved upon this method by using a Hoffman clamp by which the bulb of the medicine dropper can be more accurately compressed than with the fingers. The blood is drawn into the dropper and then, by gently screwing up the clamp, a drop is allowed to fall

* "Riforma Medica," Oct., 1896.

† "Montreal Med. Jour.," 1897.

‡ Bull. No. 5 (Sept., 1900) of the Bact. and Path. Lab. of the Delaware State Board of Health.

by its own weight upon bibulous paper, where it is dried. When the test is to be made, this dried blood-drop is dissolved in as many drops of water or other diluent as will give the necessary dilution. Unfortunately the blood quickly coagulates in the dropper.

Some have successfully employed the pipet used for counting leukocytes in making the dilutions.

Delepine suggested a method that can be employed both in the laboratory and at the bedside. A drop of blood or serum is picked up with the bacteriologist's platinum loop, placed upon a glass slide, and mixed with nine, nineteen, or thirty-nine, etc., similar drops of the culture to be used, the mixtures forming dilutions of 1 : 10, 1 : 20, 1 : 40, etc.

I * have used capillary glass tubes of equal size, which, when allowed to draw up the blood from a prick on the finger-tip, will contain a quantity of blood easily estimated from the length of the column in the tube. Knowing the quantity of contained blood, it is easy to estimate how much fluid culture one must add to make a definite dilution. The tube is crushed and stirred in the diluting culture, so that none of the blood is lost.

Hewlett and Sydney † recommend a similar, probably more exact, method.

The majority of observers use bouillon cultures of the typhoid bacillus for making the test, but I prefer fresh agar-agar cultures suspended in sterile clean water, rather than bouillon cultures, because of the larger number of bacteria they contain, the consequently greater number of agglutinations formed, and the readiness with which they are found upon microscopic examination. It is necessary, however, to make a microscopic examination before adding the serum or blood, in order to be sure that there are no natural clumps of bacteria present to simulate the specific agglutination. This is of great importance. The natural clumps of bacilli are more apt to occur in cultures grown upon fresh, moist agar-agar than upon that kept for a short time until the surface has become partially dried.

Errors are most apt to occur when too concentrated dilutions of the typhoid blood or serum are used, and it is only in cases in which sufficient dilutions of the serum produce agglutination that a positive diagnosis of typhoid can be made.

Stern ‡ concluded that the dilution should be 1 : 100, or even 1 : 200; Widal, however, found 1 : 60 sufficient. Dilutions of 1 : 50 are most satisfactory for routine work.

Welch says: "From the observations thus far reported, although they are insufficient in number for definite conclusions, there would seem to be only a small liability of failure to recognize genuine typhoid cases by resorting to dilutions of 1 : 40 or 1 : 50, but unquestionably a few would escape recognition, and for this reason lower dilutions should also be used, and if those between 1 : 10 and 1 : 50 give decided reaction there should be at least suspicion of typhoid. It is not, therefore, to be recommended that one make the test with only high dilutions, such as 1 : 50. The negative result of the preliminary test with equal parts of serum and culture suffices to exclude typhoid reaction. The examination, if positive, may then be made with a low dilution of the serum, and for this Widal's recommendation of 1 : 10 or 1 : 15 may well be adopted. If with this dilution the microscopic reaction is complete and almost immediate, as is often the case, there is prac-

* "New York Med. Jour.," Sept. 25, 1897.

† "Brit. Med. Jour.," April 28, 1900.

‡ "Centralbl. f. innere Med.," Dec. 5, 1896.

tically no risk in making a positive diagnosis. But for absolute certainty, and, above all, in cases in which the result of the reaction is not prompt, complete, and unmistakable, higher dilutions should be employed; if the amount of serum permits only one such, it may be 1 : 50; but preferably intermediate dilutions should also be made, and it is desirable, if not absolutely necessary, to try dilutions higher than 1 : 50."

A *time limit* must also be fixed, and with the standard dilution of 1 : 50 a reaction should come on in an hour.

The condition of the culture must be carefully considered from several standpoints, else the validity of the test may be called into question.

Young cultures of the typhoid bacillus are actively motile, and are apt to contain rather elongate individuals, both of which are conditions essential to success. The more actively motile the bacilli, the greater the contrast when this motility ceases; and the longer the bacilli, the more typical the agglutinations. Cultures less than twenty-four hours old are best. In an emergency, however, they can be employed so long as they remain motile (forty-eight hours).

Johnston has shown that virulent bacilli frequently transplanted from culture medium to culture medium eventually developed an abnormal activity in which reactions were apt to occur with normal blood. The best method of avoiding this error seems to be to keep a regular stock culture growing in the laboratory, transplanting it from agar to agar only as often as is necessary to keep it in good condition—say, once in three or four weeks. A culture from this to whatever medium is preferred is made about twenty-four hours before testing.

Johnston is emphatic upon the use of attenuated rather than virulent —*i. e.*, freshly isolated—cultures, stating * that "with virulent cultures the presence of agglutinative substances in non-typhoid bloods may lead to pseudo-reactions." These reactions are characterized by clumping without loss of motion. Kuhnan,† however, says that the reaction with non-virulent cultures is nearly twice as marked as that obtained with virulent ones. Foerster ‡ did not observe that the difference between the reaction obtained with virulent and attenuated bacilli was great. He experimented with nine different bacilli, and expresses the difference observed as 5 : 8.

Johnston found that when the bacilli are cultivated upon acid media they may entirely lose their ability to agglutinate. Care should be taken, therefore, to insure that the culture medium employed always has the same degree of alkalinity. On the other hand, the media must not be too alkaline, as this condition will also cause erroneous results.

Except for the fact that dead bacilli are not motile, and hence cannot show loss of motility as a part of the reactive phenomenon, they are useful for making the test. In fact, the absence of any danger of infection, and the convenience with which the sterilized cultures can be sent from place to place to be used by physicians who are unacquainted with bacteriologic technic, have made Wright and Semple § recommend their use for the purpose.

It is interesting to note the different forms of reaction, which may be described as follows:

(*a*) Cessation of motion (atypical).

* "Montreal Med. Jour.," March, 1897.

† "Berliner klin. Wochenschrift," May 10, 1897, No. 19.

‡ "Zeitschrift für Hygiene," 1897, vol. xxiv, p. 500.

§ "Brit. Med. Jour.," May 15, 1897.

(*b*) Formation of small aggregations without loss of motion of the free bacilli (atypical).

(*c*) Complete cessation of motion and formation of a reticulated agglutinated mass of bacilli covering the whole field.

(*d*) Complete cessation of motion with the formation of small, fairly uniform aggregations. This usually occurs quickly and is accompanied by distortion of the bacilli.

(*e*) Complete cessation of motion and the formation of large-sized aggregations, some of which are enormous. The bacilli are shrunken and twisted. (This form of reaction was usually almost instantaneous in its occurrence, and probably indicated that the highest degree of the blood-alteration had taken place. I saw it most marked in a case with two relapses. It is sometimes succeeded by bacteriolysis.)

(*f*) Rapid agglutination and loss of motion followed by prompt and complete solution of the bacteria. The bacteriolysis is probably entirely independent of the other phenomena. It may occur in normal blood, but few of these were examined, and the one case in which I encountered it was typical typhoid fever.

The precision of the serum diagnosis makes it of great diagnostic value. As shown by the statistics given, the reaction failed to develop in only 4.5 per cent. out of a total of 2393 cases of clinical typhoid fever, and there is little doubt but these cases were not typhoid, but paratyphoid infections.

Rumpf * and Kraus and Buswell † report a number of cases of typhoid favorably influenced by hypodermic injections of small doses of sterilized cultures of Bacillus pyocyaneus.

Following the principle of Haffkine's anticholera inoculations, Wright and Semple ‡ have used subcutaneous injections of sterilized cultures as a prophylactic measure. One cubic centimeter of a bouillon culture sterilized by heat was used.

The "Indian Medical Gazette" gives the following important figures showing what was accomplished in 1899: Among the British troops in India there were 1312 cases of typhoid fever, with 348 deaths (25 per cent.). The ratio of admissions to the total strength was 20.6 per 1000. There were 4502 inoculations and among them there were only 9 deaths from typhoid fever—0.2 per cent. of the strength. There were 44 admissions, giving 0.98 per cent. of the strength. Among the non-inoculated men of the same corps and at the same stations, of 25,851 men there were 657 cases and 146 deaths, giving the relative percentages of admissions and deaths as 2.54 and 0.56.§

* "Deutsche med. Wochenschrift," 1893, No. 41.

† "Wiener klin. Wochenschrift," July 12, 1894.

‡ "Brit. Med. Jour.," 1897, 1, p. 256.

§ "Phila. Med. Jour.," Oct. 13, 1900, p. 688.

In a later contribution, Wright* shows that this prophylactic vaccination against typhoid fever reduces the number of cases, and diminishes the death-rate among the inoculated. He also calls attention to the slight risk the inoculated run of being injured in case their vital resistance is below normal, or they are already in the early stages of the disease, or where the dose administered is too large or the second vaccination given too soon after the first.

Wright's latest paper† gives new statistics on the subject.

Walger‡ reports 4 cases treated successfully with a serum obtained from convalescent patients. Ten cubic centimeters were given at a dose, and the injection was repeated in one case with relapse.

Jez§ believes that the antitoxic principle in typhoid fever is contained in some of the internal organs instead of the blood, and claims to have obtained remarkable results in 18 cases treated with extracts of the bone-marrow, spleen, and thymus of rabbits previously injected with the typhoid bacillus.

Chantemesse,‖ Pope,** and Steele†† have all used serums from animals immunized against typhoid cultures for the treatment of typhoid fever, with more or less success. An analysis of the results will, however, show them to be very inconclusive.

The serum prepared by Macfadyen‡‡ by crushing cultures frozen with liquid air and injecting animals with the thus liberated intracellular toxin, seems to be no improvement upon others.

The chief source of error in all the serum investigations conducted up to the present time was our ignorance of the conditions under which such serums act. The early preparations were all made under the assumption that the immune typhoid serum was antitoxic. When it was more thoroughly studied, it was discovered to be bacteriolytic, but still failed to cause improvement in the treatment of the disease. We know now the conditions of bacteriolysis, and that the production of immunity in animals is accompanied by the

* "The Lancet," Sept. 6, 1902.
† "Brit. Med. Jour.," Oct. 10, 1903.
‡ "Münchener med. Wochenschrift," Sept. 27, 1898.
§ "Méd. moderne," March 25, 1899.
‖ "Gaz. des Hôpitaux," 1898, LXXI, p. 397.
** "Brit. Med. Jour.," 1897, I, 259. †† *Ibid.*, April 17, 1897.
‡‡ "Brit. Med. Jour.," April 3, 1903.

formation of an immune body only. The immune body is useless unless it be accompanied by the corresponding complementary body, and it is only by the use of some preparation containing the proper relative proportion of both substances that bacteriolysis with a favorable influence upon the course of the fever, is possible. At present we have no means of increasing the complementary body.

CHAPTER III.

BACILLI RESEMBLING THE TYPHOID BACILLUS.

Bacillus typhosus is one of a group of organisms possessing a considerable number of common characteristics, each member of which, however, can be differentiated by some one fairly well-marked peculiarity. At one end of the series is the typhoid bacillus, of which we conceive as devoid of the power to ferment sugars, form indol, coagulate milk, or progressively form acids. At the other extreme stands Bacillus coli communis, an organism whose typical representatives coagulate milk, form indol, ferment dextrose, lactose, saccharose, and maltose with the formation of hydrogen and carbon dioxid in the proportion of $\frac{H}{CO_2} = \frac{2}{1}$.

Between these extremes are numerous organisms known as "intermediates." It is usually a simple matter to differentiate these forms from the typical species at the two ends of the series, but it is quite difficult to differentiate them from one another. Whether they are of sufficient importance to make it worth while to pay much attention to them is, as yet, uncertain; and, indeed, we do not know whether they are to be regarded as variations from the type species or separate and distinct organisms. The fact that some of them are associated with serious and fatal disorders—paracolon and psittacosis—suggests that they have an importance that is just becoming recognized.

In his careful review of the intermediate forms thus far described, Buxton * summarizes the main points of difference as follows:

	B. Coli Communis.	Intermediates.	B. Typhosus.
Coagulation of milk	+	—	—
Production of indol	+	—	—
Fermentation of lactose with gas	+	—	—
Fermentation of glucose with gas	+	+	—
Agglutination by typhoid serum	—	—	+

* "Journal of Medical Research," vol. viii, No. 1, June, 1902, p. 201.

Buxton finds those pathogenic for man clinically divisible into three groups, as follows:

(*a*) *The Meat-poisoning Group.*—This includes Bacillus enteritidis of Gärtner and others. The symptoms begin soon after eating the poisonous meat, and are toxic. Bacilli quickly invade the body. The illness continues four or five days, after which recovery is quick. In a few cases death has occurred on the second or third day.

(*b*) *The Pneumonic or Psittacosis Group.*—Psittacosis is an epidemic infectious disease with pneumonic symptoms and a high mortality. Its origin has been traced to diseased parrots, and from them Nocard isolated Bacillus psittacosis, supposed to be the cause of the disease in man. Later epidemics were studied by Achard and Bensaude.

(*c*) *The Typhoidal Group.*—The organisms to be included in this group occasion symptoms closely resembling typhoid fever, though they differ biologically from the typhoid bacillus, and do not agglutinate with typhoid serums.

It is thus evident that some of the intermediates occasion symptoms resembling typhoid fever, while others occasion symptoms widely differing from it. It is suggested that to the former the term *paratyphoid bacilli* be applied, while the latter are known as *paracolon bacilli.*

Although Achard and Bensaude,* and Johnson, Hewlett, and Longcope † have studied the paratyphoid infections, Gwyn,‡ Libman,§ and others the paracolon bacilli, and Cushing‖ and Durham ** have made comparative studies of the members of the group, it is still too soon to regard the knowledge attained sufficient to warrant particular mention of the various intermediate and related organisms in a work of this kind. In the following pages, therefore, attention will be devoted only to the more important organisms of the group and to a few belonging to offshoots from the parent stem—Bacillus dysenteriæ, Bacillus fæcalis alkaligenes, and Bacillus psittacosis.

* "Soc. Med.," Nov., 1896.
† "Amer. Jour. Med. Sci.," Aug., 1902.
‡ "Johns Hopkins Bulletin," vol. IX, 1898.
§ "Journal of Medical Research," 1902, p. 168.
‖ "Johns Hopkins Bulletin," vol. XI, 1900.
** "Journal of Experimental Medicine," vol. V, p. 353, 1901.

Bacillus Coli Communis (Escherich).

General Characteristics.—A motile, flagellated, non-sporogenous, aerobic and optionally anaerobic, non-chromogenic, non-liquefying, aerogenic, saprophytic, occasionally pathogenic bacillus, staining by the ordinary methods, but not by Gram's method.

This micro-organism was first isolated from human feces by Emmerich,* in 1885, who thought it to be the specific cause of Asiatic cholera, and called it Bacillus neapolitanus. Many investigators have since studied its peculiarities, until it has become one of the best known bacteria.

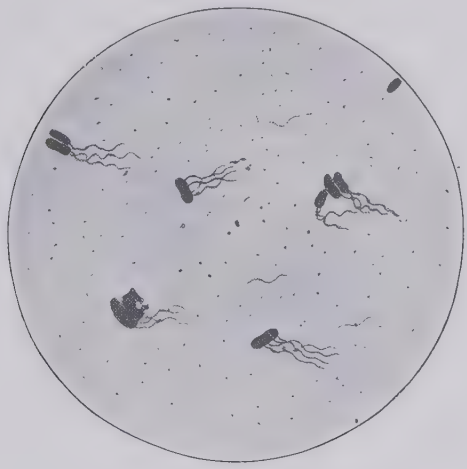


Fig. 152.—Bacillus coli (Migula).

Distribution.—It is habitually present in the feces of animals, and in water and soil contaminated with them. Soon after birth the organism finds its way into the alimentary canal and permanently establishes itself in the intestine, where it can be found in great numbers throughout the entire life of the individual. It is almost certainly identical with Bacillus pyogenes fœtidus of Passet, and so closely resembles B. acidi lactici that Prescott † believes them to be identical. It may also be identical with Bacillus lactis aerogenes, Bacillus cavicida, and other described species.

* "Deutsche med. Wochenschrift," 1885, No. 2.

† Society of American Bacteriologists, Dec. 31, 1902.

Morphology.—The bacillus is rather variable, both size and form depending to a certain extent upon the culture medium on which it grows. It measures about 1–3 × 0.4–0.7 μ. It usually occurs in the form of short rods, but coccus-like individuals and elongate individuals may be found in the same culture. The bacilli are usually separate from one another, though occasionally joined in pairs, are actively motile, and provided with flagella, which are variable in number, usually from four to a dozen. The organisms from some cultures swim actively, even when the culture is some days old; others are sluggish even when young

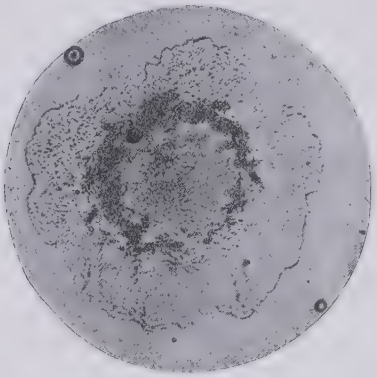


Fig. 153.—Bacillus coli communis; superficial colony two days old upon a gelatin plate. × 21 (Heim).

and actively growing, and still other cultures consist of bacilli that scarcely move at all. It forms no endospores.

Staining.—The bacillus stains well with the aqueous solutions of the anilin dyes, but not by Gram's method.

Cultivation.—It is readily cultivated upon the ordinary media.

Colonies.—Upon gelatin plates the colonies are visible in twenty-four hours. Those situated below the surface appear round, yellow-brown, and homogeneous. As they increase in size they become opaque. The superficial colonies are larger and spread out upon the surface. The edges are dentate and slightly resemble grape-vine leaves, often showing radiating ridges suggestive of the veins of a leaf. They may have a slightly concentric appearance.

The colonies rapidly increase in size and become more and more opaque. The gelatin is not liquefied.

Gelatin Punctures.—In gelatin punctures development upon the surface, and also in the needle's track, causes the formation of a nail-like growth. The head of the nail may reach the walls of the test-tube. Gas may be formed in ordinary gelatin, and when 1 per cent. of dextrose is dissolved in the medium, the gas-production is often so copious and rapid as to form large bubbles, which subsequently break it up irregularly. The gelatin may become slightly clouded as the bacilli grow, but is not liquefied.

Agar-agar.—Upon agar-agar along the line of inoculation a grayish-white, translucent, smeary growth devoid of any characteristics takes place. The entire surface of the culture medium is never covered, the growth remaining confined to the inoculation line, except where the moisture of the condensation fluid allows it to spread out at the bottom. Kruse says that crystals may form in old cultures.

Bouillon.—Bouillon is densely clouded by the growth of the bacteria, a delicate pellicle at times forming upon the surface. There is usually considerable sediment in the culture.

Potato.—Upon potato the growth is luxuriant. The bacillus forms a yellowish-brown, glistening layer spreading from the line of inoculation over about one-half to two-thirds of the potato. The color varies considerably, sometimes being pale, sometimes quite brown, sometimes greenish. It cannot, therefore, be taken as a characteristic of much importance. The growth on potato may be almost invisible.

Milk.—In milk rapid coagulation and acidulation occur, with the evolution of gas. The culture gives off a fecal odor. Litmus added to the culture media is first reddened, then decolorized by the bacilli.

Vital Resistance.—It is quite resistant to antiseptics and germicides, and grows in culture media containing from 0.1–0.2 per cent. of carbolic acid. It is, however, easily killed by heat, and is destroyed by exposure to 60° C. for ten minutes.

Metabolic Products.—Würtz found that Bacillus coli produced ammonia in culture media free from sugar, and thus caused an intense alkaline reaction in the culture media. The cultures usually give off an odor that varies somewhat, but is, as a rule, unpleasant.

Nitrates are reduced to nitrites by the growth of the bacillus.

In bouillon containing 1 per cent. of dextrose, lactose, and saccharose, the colon bacillus splits up the sugar, liberating CO_2 and H, the gas formula being $\frac{H}{CO_2} = \frac{2}{1}$. This gas formula is very constant for the micro-organisms of the colon group and forms one of their most important differential characteristics. In sugar-containing bouillon acetic, lactic, and formic acids are produced.

The bacillus requires very little nutriment. It grows in Uschinsky's asparagin solution, and is frequently found living in river and well waters.

Indol is formed in both bouillon and peptone solutions, but phenol is not produced. The presence of indol is best determined by Salkowski's method (*q. v.*).

Toxic Products.—Vaughan and Cooley* have shown that the toxin of the colon bacillus is contained in the germ-cell and under ordinary conditions does not diffuse from it into the culture medium. The toxin may be heated in water to a very high temperature without injuring its poisonous nature. They have devised an apparatus in which enormous cultures can be prepared and the bacteria pulverized.† Of such a preparation 0.0002 gram will kill a 200-gram guinea-pig.

Pathogenesis.—The bacillus begins to penetrate the intestinal tissues almost immediately after death, and is the most frequent contaminating micro-organism met with in cultures made at autopsy. It may spread by direct continuity of tissue, or *via* the blood-vessels.

Although under normal conditions a saprophyte, the colon bacillus is not infrequently found in the pus in suppurations connected with the intestines—as, for example, appendicitis—and sometimes in suppurations remote from them.

In intestinal diseases, such as typhoid, cholera, and dysentery, the bacillus not only seems to acquire an unusual degree of virulence, but because of the existing denudation of mucous surfaces, etc., finds it easy to enter the general system, with the formation of remote secondary suppurative lesions in which it is the essential factor. When ab-

* "Jour. Amer. Med. Assoc.," 1901; and "American Medicine," 1901.

† "Trans. Assoc. Amer. Phys.," 1901.

sorbed from the intestine, it frequently enters the kidney and is excreted with the urine, causing, incidentally, local inflammatory areas in the kidney, and occasionally cystitis. A case of urethritis is reported to have been caused by it.

In infants cholera infantum may not infrequently be caused by the colon bacillus, though probably in this disease other bacteria play an important rôle (B. dysenteriæ ?).

The bile-ducts are sometimes invaded by the bacillus, which may lead to inflammation, obstruction, suppuration, or calculous formation.

The colon bacillus has also been met with in puerperal fever, Winckel's disease of the new-born, endocarditis, meningitis, liver-abscess, broncho-pneumonia, pleuritis, chronic tonsillitis, and urethritis.

Virulence.—It is a question whether the colon bacillus is always virulent, or whether it becomes so under abnormal conditions. Klencki * found it very virulent in the ileum, and less so in the colon and jejunum of dogs. He also found that the virulence was greatly increased in a strangulated portion of intestine. Dreyfus † found that the colon bacillus as it occurs in normal feces is not virulent. Most experimenters believe that pathologic conditions, such as disease of the intestine, ligation of the intestine, etc., increase its virulence.

Frequent transplantation lessens the virulence of the bacillus; passage through animals increases it.

It has been observed that cultures of the bacillus obtained from cases of cholera, cholera nostras, and other intestinal diseases are more pathogenic than those obtained from normal feces or from pus.

Adelaide Ward Peckham,‡ in an elaborate study of the "Influence of Environment on the Colon Bacillus," concludes that while the conditions of nutrition and development in the intestine seem to be most favorable, the colon bacillus is ordinarily not virulent. She says:

"Its first force is spent upon the process of fermentation, and as long as opportunities exist for the exercise of this function the affinities of this organism appear to be strongest in this direction.

"Moreover, the contents of the intestine remain acid until they

* "Ann. de l'Inst. Pasteur," 1895, No. 9.

† "Centralbl. f. Bakt.," etc., XVI, p. 581.

‡ "Journal of Experimental Medicine," Sept., 1897, vol. II, No. 4, p. 549.

reach the neighborhood of the colon, and by that time the tryptic peptones have been formed and absorbed to a great extent.

"During the process of inflammation in the digestive tract a very different condition may exist. The peptic and tryptic enzymes may be partially suppressed. Fermentation of carbohydrates and proteid foods then begins in the stomach, and continues after the mass of food is passed on into the intestine. The colon bacillus cannot, therefore, spend its force upon fermentation of sugars, because they are already broken up and an alkaline fermentation of the proteids is in progress. It also cannot form peptones from the original proteids, for it does not possess this property, and unless trypsin is present it must be dependent upon the proteolytic activity of other bacteria for a suitable form of proteid food. Perhaps these bacteria form an albuminate molecule which, like leucin and tyrosin, cannot be broken up into indol, and thus there might be caused an important modification of the metabolism of the colon bacillus, which might have either an immediate or remote influence upon its acquisition of disease-producing properties, for our own experiments indicate that the power to form indol, and the actual forming of it, are to some extent an indication of the possession of pathogenesis."

For the laboratory animals the colon bacillus is pathogenic in varying degree. Intraperitoneal injections into mice cause death in from one to eight days if the culture be virulent. Guinea-pigs and rabbits also succumb to intraperitoneal and intravenous injection. Subcutaneous injections are of less effect, and in rabbits seem to produce abscesses only.

When injected into the abdominal cavity, the bacilli set up a sero-fibrinous or purulent peritonitis, and are very numerous in the abdominal fluids.

Cumston,* from a careful study of thirteen cases of summer infantile diarrheas, comes to the following conclusions:

Bacterium coli seems to be the pathogenic agent of the greater number of summer infantile diarrheas.

The organism is often associated with Streptococcus pyogenes.

The virulence, more considerable than in the intestine of a healthy child, is almost always in direct relation to the condition of the child at the time the culture is taken, and does not appear to be proportionate to the ulterior gravity of the case.

The mobility of Bacterium coli is, in general, proportionate to its virulence. The jumping movement, nevertheless, does not correspond to an exalted virulence in comparison with the cases in which the mobility was very considerable, without presenting these jumping movements.

The virulence of Bacterium coli found in the blood and other organs is identical with that of Bacterium coli taken from the intestines of the same individual.

Lesage,† in studying the enteritis of infants, found that

* "International Medical Magazine," Feb., 1897.

† "La Semaine médicale," Oct. 20, 1897.

in 40 out of 50 cases depending upon Bacillus coli the blood of the patient agglutinated the cultures obtained, not only from his own stools, but from those of all the other cases. From this uniformity of action Lesage suggests that the colon bacilli in these cases are all of the same species.

The agglutinating reaction occurs only in the early stages and acute forms of the disease.

Immunization.—It is not difficult to immunize an animal against the colon bacillus. Löffler and Abel immunized dogs by progressively increased subcutaneous doses of live bacteria, grown in solid culture and suspended in water. The injections at first produced hard swellings. The blood of the immunized animals possessed an active bactericidal effect upon the colon bacteria. The serum was not in the correct sense antitoxic.

Differential Diagnosis.—This problem is considered at greater length under the differential diagnosis of the Bacillus typhosus (*q. v.*). For the recognition of the colon bacillus the most important points are the motility, the indol formation, the milk-coagulation, and the active gas-production. As, however, all of these features are shared by other bacteria to a greater or less degree, the most accurate differential point is the immunity reaction with the serum of an immunized animal, which protects susceptible animals from the effects of inoculation, and produces a similar agglutinative reaction to that observed in connection with the blood and serum of typhoid patients, convalescents, and immunized animals.

The fact that, with rare exceptions, the typhoid serum produces a specific reaction with the typhoid bacillus, and the colon serum with the colon bacillus, should be the most important evidence that they are entirely different species.

I have no doubt that what is commonly known as Bacillus coli communis is not a single species, but a name at present applied to a group of bacilli too similar to be differentiated by our present methods. This opinion seems to be shared by others, and a separation into groups, types, or families has been attempted.

In order to establish a *type species* of Bacillus coli communis, Smith * says:

"I would suggest that those forms be regarded as true to this species which grow on gelatin in the form of delicate bluish or more opaque,

* "Amer. Jour. Med. Sci.," 1895, 110, p. 287.

whitish expansions with irregular margin, which are actively motile when examined in the hanging drop from young surface colonies taken from gelatin plates, which coagulate milk within a few days; grow upon potato, either as a rich pale or brownish-yellow deposit, or merely as a glistening, barely recognizable layer, and which give a distinct indol reaction. Their behavior in the fermentation-tube must conform to the following scheme:

"*Variety α:*

"One per cent. dextrose-bouillon (at 37° C.). Total gas approximately $\frac{1}{2}$; H — CO_2 = approximately 2 : 1; reaction strongly acid.

"One per cent. lactose-bouillon: as in dextrose-bouillon (with slight variations).

"One per cent. saccharose-bouillon; gas-production slower than the preceding, lasting from seven to fourteen days. Total gas about $\frac{2}{3}$; H — CO_2 = nearly 3 : 2. The final reaction in the bulb may be slightly acid or alkaline, according to the rate of gas-production.

"*Variety β:*

"The same in all respects, excepting as to its behavior in saccharose-bouillon; neither gas nor acids are formed in it."

DIFFERENTIAL CHARACTERISTICS.

Typhoid Bacillus.	Colon Bacillus.
Bacilli usually slender.	Bacilli a little thicker and shorter.
Flagella numerous (10–20), long, and wavy (peritricha).	Flagella fewer (8–10) (peritricha).
Growth not very rapid, not particularly luxuriant.	Growth rapid and luxuriant. This character is by no means constant.
Upon Elsner's, Hiss', Piorkowski's, and other media gives characteristic appearances.	Upon Elsner's, Hiss', Piorkowski's, and other media gives characteristic appearances.
Upon fresh acid potato the so-called "invisible growth" formerly thought to be differential.	Upon potato a brownish-yellow distinct pellicle.
Acid-production in whey not exceeding 3 per cent. Sometimes slight in ordinary media, and succeeded by alkali-production.	Acid-production well marked throughout.
Grows in media containing sugars without producing any gas.	Fermentation with gas-production well marked in solutions containing dextrose, lactose, or saccharose, the usual formula being H — CO_2 = 2 : 1.
Produces no indol.	Indol-production marked.
Growth in milk unaccompanied by coagulation.	Milk coagulated.
Gives the Widal reaction with the serum of typhoid blood.	Does not react with typhoid blood.

Bacillus Enteritidis (Gärtner).

General Characteristics.—A motile, flagellated, non-sporogenous, non-chromogenic, non-liquefying, aerobic and optionally anaerobic, pathogenic bacillus staining by the ordinary methods, but not by Gram's method.

This bacillus was first cultivated by A. Gärtner* from the flesh of a cow slaughtered because of an intestinal disease, and from the spleen of a man poisoned by eating meat obtained from it. The bacillus was subsequently found by Karlinski and Lubarsch in other cases of meat-poisoning.

Morphology.—The bacillus closely resembles Bacillus coli communis. It is short and thick, is surrounded by a slight capsule, is actively motile, and has flagella.

Staining.—It stains irregularly with the ordinary solutions, but not by Gram's method. It has no spores.

Cultivation.—Upon gelatin plates it forms round, pale gray, translucent colonies. It does not liquefy the gelatin. The deep colonies are brown and spheric. The growth on agar-agar is similar to that of the colon bacillus. The organism produces no indol, coagulates milk in a few days, and reduces litmus. Its fermentative powers have not been sufficiently studied. Upon potato it forms a yellowish-white, shining layer.

Pathogenesis.—The bacillus is pathogenic for mice, guinea-pigs, pigeons, lambs, and kids, but not for dogs, cats, rats, or sparrows. The infection may be fatal for mice and guinea-pigs, whether given subcutaneously, intraperitoneally, or by the mouth.

Lesions.—The bacilli are found scattered throughout the organs in small groups, resembling those of the typhoid bacillus.

At the autopsy a marked enteritis and swelling of the lymphatic follicles and patches, with occasional hemorrhages, are found. The bacilli occur in the intestinal contents. The spleen is somewhat enlarged.

The bacillus is differentiated from the colon bacillus chiefly by the absence of indol-production, by its ability to produce infection when ingested, and by the fact that it elaborates a toxic substance capable of producing symptoms similar to those seen in the infection.

It may be distinguished from Bacillus lactis aerogenes by its motility. It is with great difficulty separable from certain water bacteria; but so far as is known its pathogenesis can be made use of for assisting in its differentiation in doubtful cases.

* "Korrespond. d. allg. ärztl. Ver. von Thüring," 1888, 9.

Bacillus Dysenteriæ (Shiga).

General Characteristics.—A motile, flagellated, non-sporogenous, non-liquefying, aerobic and optionally anaerobic, non-chromogenic, non-aerogenic, pathogenic bacillus of the intestine, staining by ordinary methods, but not by Gram's method.

After considerable investigation of the epidemic dysentery prevalent in Japan, Shiga * has come to the conclusion that a bacillus which he calls Bacillus dysenteriæ is its specific cause.

It is not improbable that the bacillus of Shiga is identical with *Bacterium coli*, variety *dysenteriæ*, of Celli, Fioca, and Scala,† a view that has been further confirmed by Flexner.‡ It may also be identical with an organism described in 1888 by Chantemesse and Widal.§

Morphology.—The organism is a short rod, very similar to the typhoid and colon bacilli. It is feebly motile.

Staining.—When stained with methylene-blue the ends color more deeply than the middle; and organisms from old cultures show numerous involution forms and irregularities. It stains with ordinary solutions, but not by Gram's method. It has no spores, but has flagella (peritricha).

Cultivation.—Colonies.—The colonies upon gelatin plates are small and dewdrop-like in appearance. Upon microscopic examination they are seen to be regular and of spheric form. By transmitted light they appear granular and of a yellowish color. They do not spread out in a thin pellicle like those of the colon bacillus, and there are no essential differences between superficial and deep colonies.

Gelatin Punctures.—The growth in the puncture culture consists of crowded, rounded colonies along the puncture. A grayish-white growth forms upon the surface. There is no liquefaction of the gelatin.

Agar-agar.—Upon the surface of agar-agar cultures kept in the incubating oven, large, solitary colonies are evident at the end of twenty-four hours. They are bluish-white in color and rounded in form. The surface appears moist. In the course of forty-eight hours a transparent

* "Centralbl. f. Bakt. u. Parasitenk.," 1898, xxiv, Nos. 22–24.

† "Hygien. Institut. Rom. Univ.," 1895, and "Centralbl. f. Bakt. u. Parasitenk.," 1899.

‡ "Univ. of Penna. Med. Bulletin," Aug., 1901.

§ See "Deutsche med. Wochenschrift," 1903, No. 12.

border is observed about each colony, and the bacilli of which it is composed cease to stain evenly, presenting involution forms.

Glycerin agar-agar seems less well adapted to their growth than plain agar-agar. Blood-serum is not a suitable medium.

Potato.—Upon boiled potato the young growth resembles that of the typhoid bacillus, but after twenty-four hours it becomes yellowish-brown, and at the end of a week forms a thick, brownish-pink pellicle.

Bouillon.—In bouillon the bacillus grows well, clouding the liquid. No pellicle forms on the surface.

Metabolic Products.—The organism does not form indol, does not ferment sugar, and in gelatin and agar-agar containing sugars no gas is evolved. Acids are produced in moderate quantities after twenty-four hours. Milk is not coagulated.

Vital Resistance.—Its thermal death-point is 68° C. maintained for twenty minutes. It grows slowly at ordinary temperatures, rapidly at the temperature of the body. The culture media should be alkaline.

Pathogenesis.—Bacillus dysenteriæ was found by Shiga in all the cases of epidemic dysentery studied in Japan, by Flexner in the epidemic dysentery of the Philippine Islands, and by Vedder and Duval * in the epidemic and sporadic dysentery of the United States. Duval and Bassett† and Martha Wollstein‡ found Bacillus dysenteriæ in cases of the summer diarrheas of infants, especially when such diarrheas were epidemic.

Diagnosis.—Kazarinow § found that when guinea-pigs and young rabbits were narcotized with opium, the gastric contents alkalinized with 10 c.c. of a 10 per cent. NaOH solution, and a quantity of Shiga bacilli introduced into the stomach with an esophageal bougie, it was possible to bring about diarrhea and death with lesions similar to those described by Vaillard and Dopter.

In these experiments it was found that rapid passage

* "Journal of Experimental Medicine," vol. VI, No. 2, 1902; and "American Medicine," 1902.

† "American Medicine," Sept. 13, 1902, vol. IV, No. 11, p. 417.

‡ "Jour. Med. Research," X, p. 11, 1904.

§ "Archiv. f. Hyg.," Bd. L, Heft 1, p. 66; see also "Bull. de l'Inst. Past.," 15 Aout, 1904, p. 634.

through animals greatly increased the virulence of the bacilli, and it was also observed that though 0.0005 c.c. of a virulent culture introduced into the peritoneal cavity would cause fatal infection, to produce infection by the mouth as above stated required the entire mass of organisms grown in five whole culture tubes.

Eyre* has suggested the following method for the examination of the fecal material and scrapings from dysenteric ulcers, the work of differentiation and isolation being facilitated by the employment of a special nutrient medium—a modified form of the "lakmus-laktose-nutrose-agar" of Drigalski and Conradi. A platinum loop, reserved for this particular purpose, and capable of holding about 2 mg. of feces, was employed to emulsify three or more loopfuls of the specimen in 10 c.c. of sterile nutrient broth. Three loopfuls of this suspension were deposited on the surface of a nutrose agar "plate," and then spread evenly over its entire surface by means of a sterile L-shaped glass rod. Two, and sometimes three, other plates were then inoculated in series, and the whole set of plates incubated at 37° C. for twenty-four hours. At the end of this time such colonies as corresponded in their naked-eye appearances, blue coloration, and characteristic odor of fresh semen, with those produced by a typical B. dysenteriæ (Shiga) were severally emulsified with sterile broth, and tested against a 1 : 100 and a 1 : 200 dilution of antidysenteric serum (for which I am indebted to Professor Kolle, of the Institute für Infektionskrankheiten, Berlin) both macroscopically and microscopically.

If a good agglutination reaction was obtained within half an hour the organism was grown upon agar, the subsequent growth plated, the organism then isolated and subcultivated in parallel series with an authentic B. dysenteriæ (also obtained from Professor Kolle), and its identity or otherwise therewith determined.

The blood-serum of those suffering from epidemic dysentery or from those recently recovered from it causes a well-marked agglutinative reaction. This agglutination has been carefully studied by Flexner, and is thought to be specific and useful for diagnosticating the disease.

By the progressive immunization of horses to an immunizing fluid, the basis of which is a twenty-four-hour-old agar-agar culture dried *in vacuo*, Shiga has prepared an antitoxic serum with which, in 1898, in the Laboratory Hospital, 65 cases were treated, with a death-rate of 9 per cent.; in 1899, in the Laboratory Hospital, 91 cases, with a death-rate of 8 per cent.; in 1899, in the Hirowo Hospital, 110 cases, with a death-rate of 12 per cent. These results are very significant, as the death-rate in 2736 cases simultaneously treated without the serum averaged 34.7 per cent., and in consideration of the frequency and high death-rate of the disease, Japan alone, between the years

* "Brit. Med. Jour.," April 30, 1904, p. 1002.

1878 and 1899, furnishing a total of 1,136,096 cases, with 275,308 deaths (a total mortality for the entire period of 24.23 per cent.).*

The "epidemic dysentery" is not the same affection as "amebic dysentery," the chief points of dissimilarity being that the extensive undermined ulcerations, described by Councilman and Lafleur, as so characteristic of the latter, are rarely observed. The follicular, pustule-like ulcer is very uncommon; perforation is unusual, the muscular coat offering strong resistance to the disease process. Abscess of the liver is also rare in epidemic, though common in amebic dysentery.

Pseudo-dysentery Bacillus.

Kruse † has observed in dysenteric diseases of the insane a new (?) bacillus which he has called the *pseudo-dysentery bacillus*. The morphologic and cultural differences between it and B. dysenteriæ are slight, but the agglutination reactions are quite different and form the ground for separation.

Bacillus Fæcalis Alkaligenes (Petruschky).

General Characteristics.—A motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, non-aerogenic, aerobic and optionally anaerobic, non-pathogenic bacillus of the intestine, staining by ordinary methods, but not by Gram's method.

This bacillus has occasionally been isolated by Petruschky ‡ and others from feces. It closely resembles the typhoid bacillus, being short, stout, with round ends, forming no spores, staining with the usual dyes, but not by Gram's method, being actively motile, and having numerous flagella. It does not liquefy gelatin, does not coagulate milk, produce gas, or form indol. Its pathogenic powers are similar to those of the typhoid bacillus.

It grows more luxuriantly than the typhoid bacillus upon potato, producing a brown color, and generates a strong alkali when grown in litmus-whey. Its cultures are not agglutinated by the typhoid serums.

* "Public Health Reports," Jan. 5, 1900, vol. xv, No. 1.

† "Deutsche med. Wochenschrift," 1901, Nos. 23 and 24.

‡ "Centralbl. f. Bakt. u. Parasitenk.," xix, 187.

Bacillus Psittacosis (Nocard).

General Characteristics.—A motile, flagellated, non-sporogenous, aerobic, optionally anaerobic, non-chromogenic, aerogenic, pathogenic, non-liquefying bacillus, staining by the ordinary methods, but not by Gram's method.

This micro-organism was discovered by Nocard,* who first observed it in 1892 in certain cases of psittacosis, or epidemic pneumonia traceable to infection from diseased parrots. The original paper contained an excellent account of the specific organism.

The subsequent work of Gilbert and Fournier† shows the specificity of the micro-organism to be quite well established and Nocard's characterizations accurate.

Morphology.—The bacillus is short, stout, rounded at the ends, and actively motile. It is provided with flagella, but forms no spores. It resembles the typhoid and the colon bacilli and is evidently a form intermediate between the two.

Isolation.—Gilbert and Fournier succeeded in isolating it from the blood of a patient dead of psittacosis, and from parrots by the use of lactose-litmus-agar. The organism does not alter the litmus, and if a small percentage of carbolic acid be added to the culture media, it grows as does the typhoid bacillus.

Cultivation.—The colonies, agar-agar and gelatin cultures, closely resemble those of the typhoid fever organism. Upon potato it more closely resembles the colon bacillus. Bouillon becomes clouded.

Metabolic Products.—In bouillon containing sugars the micro-organism is found to ferment dextrose, but not lactose. Milk is not coagulated and not acidulated. No indol is formed.

Pathogenesis.—Bacillus psittacosis can be immediately differentiated from the typhoid and colon bacilli by its peculiar pathogenesis. It is extremely virulent for parrots, producing a fatal infection in a short time. White and gray mice and pigeons are equally susceptible. Ten drops of a bouillon culture injected in the ear-vein of a rabbit

* Séance du Conseil d'hygiene publique et Salubrite du Departement de la Seine, March 24, 1893.

† "Comptes de la Société de Biologie," 1896; and "La Presse médicale," Jan. 16, 1897.

kill it in from twelve to eighteen hours. Guinea-pigs are more resistant. Subcutaneous injection of dogs produces a hard, painful swelling, which persists for a short time and then disappears without suppuration. It is also infectious for man, a number of epidemics of peculiar pneumonia, characterized by the presence of the bacillus in the blood, traceable to diseased parrots, having been reported.

Differentiation.—Bacillus psittacosis can best be differentiated from the typhoid and the colon bacilli and others of the same group by its pathogenesis and by the reaction of agglutination. Typhoid immune serum produces some small agglutinations, but a comparison between these and the agglutinations formed by cultures of the typhoid bacillus shows immediately that the micro-organisms are dissimilar. Differentiation is best made out when the prepared hanging-drop specimens of serums and cultures are kept for some hours in an incubating oven. It is not known whether the bacillus is peculiar to the intestines of parrots, invading their tissues when they become ill, or whether it is a purely pathogenic micro-organism found only in psittacosis.

CHAPTER IV.

YELLOW FEVER.

THE bacteriology of yellow fever has been studied by Domingos Freire,* Carmona y Valle,† Sternberg,‡ Havelburg,§ and Sanarelli,‖ but all of their work has been shown to be incorrect by the interesting researches and very conclusive results of Finlay,** Carter,†† Reed, Carroll, Lazear, and Agramonte,‡‡ and Reed and Carroll,§§ which have proved the mosquito to be the definitive host of a parasite probably belonging to the animal kingdom. Two of the described bacteria, however, deserve mention, not because they have much probable connection with yellow fever, but because of certain striking peculiarities they possess.

BACILLUS X (STERNBERG).

Sternberg‖‖ reported the study of 42 yellow fever autopsies in which aerobic and anaerobic cultures were made from the blood, liver, kidney, urine, stomach, and intestines, but the specific infectious agent was not found, and the most approved bacteriologic methods failed to demonstrate the

* "Doctrine microbienne de la fievre jaune et ses inoculation preventives," Rio Janeiro, 1885.

† "Leçons sur l'étiologie et la prophylaxie de la fievre jaune," Mexico, 1885.

‡ "Report on the Etiology and Prevention of Yellow Fever," Washington, 1891; "Report on the Prevention of Yellow Fever by Inoculation," Washington, 1888.

§ "Ann. de l'Inst. Pasteur," 1897.

‖ "Brit. Med. Jour.," July 3, 1897, and "Ann. de l'Inst. Pasteur," June, Sept., and Oct., 1897.

** "Amer. Jour. Med. Sci.," 1891, vol. CII. p. 264; "Ann. de la Real Academia," vol. XVIII, 1881, p. 147–169; "Jour. Amer. Med Assoc.," vol. XXXVIII, April 19, 1902, p. 993.

†† "New Orleans Med. Jour.," May, 1890.

‡‡ "Phila. Med. Jour.," Oct. 27, 1900; "Public Health," vol. XXVI 1900, p. 23.

§§ "Public Health," vol. XXVII, 1901, p. 113.

‖‖ Tenth International Medical Congress, Berlin, 1890.

constant presence of any particular micro-organism in the blood and tissues of yellow fever cadavers. The micro-organism most frequently encountered was Bacillus coli communis.

The most interesting micro-organism met with was Bacillus *x*, which he isolated from a considerable number of cases, and may have been present in all. It was not present in any of the control experiments. It was very pathogenic for rabbits when injected into the abdominal cavity. Sternberg says: "It is possible that this bacillus is concerned in the etiology of yellow fever, but no satisfactory evidence that this is the case has been obtained by experiments upon the lower animals, and it has not been found in such numbers as to warrant the inference that it is the veritable infectious agent." It is so similar to Bacillus icteroides that they may be identical.

Bacillus Icteroides (Sanarelli).

General Characteristics.—An actively motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, aerogenic, aerobic and optionally anaerobic, pathogenic bacillus which stains by the ordinary method, but not by Gram's method.

Sanarelli regarded this bacillus as the specific organism of yellow fever. He found it in 11 autopsies upon yellow fever cases, but always found it associated with streptococci, colon bacilli, proteus, and other organisms. It is found in the blood and tissues, and not in the gastro-intestinal tract, and by proper methods isolation of the organism was possible in only 58 per cent. of the cases, and in rare instances was accomplished during life.

Distribution.—By suitable methods it can be found in the organs of yellow fever cadavers, usually aggregated in small groups, in the capillaries of the liver, kidneys, and other organs. The best method of demonstration is to keep a fragment of liver, obtained from a body soon after death, in the incubator at 37° C. for twelve hours and allow the bacteria to multiply in the fresh tissue before examination.

Morphology.—The bacillus presents nothing morphologically characteristic. It is a small pleomorphous bacillus with rounded ends, usually joined in pairs. It is 2–4 μ in length, and, as a rule, two or three times longer than broad

(Fig. 154). It is actively motile and has flagella. It does not form spores.

Staining.—It stains by the usual methods, but not by Gram's method.

Cultivation.—The bacillus can be grown upon the usual media. It grows readily at ordinary room temperatures, but best at 37° C.

Colonies.—Upon gelatin plates it forms rounded, transparent, granular colonies, which during the first three or four days somewhat resemble leukocytes. The granular appearance becomes continuously more marked, and usu-

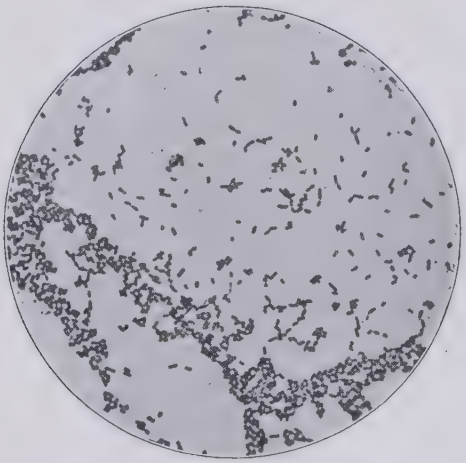


Fig. 154.—Bacillus icteroides (Sanarelli).

ally an opaque central or peripheral nucleus is seen. In time the entire colony becomes opaque, but does not liquefy gelatin.

Gelatin.—Stroke cultures on obliquely solidified gelatin show brilliant, opaque, white colonies resembling drops of milk.

Bouillon.—In bouillon it develops slowly, without either pellicle or flocculi.

Agar-agar.—The culture upon agar-agar is said to be characteristic.

The peculiar and characteristic appearances of the colonies do not develop if grown at 37° C.; but at 20°–22° C. the

colonies appear rounded, whitish, opaque, and prominent, like drops of milk. This appearance of the colonies also shows well if the cultures are kept for the first twelve to sixteen hours at 37° C., and afterward at the room temperature, when the colonies will show a flat central nucleus, transparent and bluish, surrounded by a prominent and

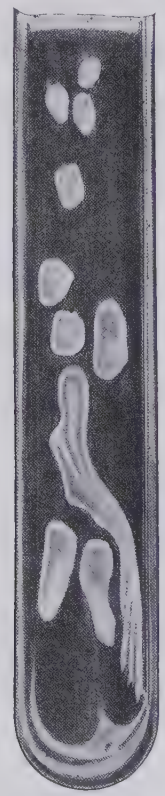


Fig. 155.—Culture of bacillus icteroides on agar (Muir and Ritchie).

opaque zone, the whole resembling a drop of sealing-wax. Sanarelli refers to this appearance as constituting the chief diagnostic feature of Bacillus icteroides. It can be observed in twenty-four hours.

Blood-serum.—Upon blood-serum the growth is very meager.

Potato.—The growth upon potato corresponds to the classic description of that of the bacillus of typhoid fever.

Vital Resistance.—It strongly resists drying, but dies when exposed in cultures to a temperature of 60° C. for a few minutes, and is killed in seven hours by the solar rays. It can live for a considerable time in sea-water.

Metabolism.—The bacillus is an optional anaerobe. It slowly ferments dextrose, lactose, and saccharose, forming gas only in dextrose solutions in which there are no other sugars. It does not coagulate milk. In the cultures a small amount of indol is formed.

Pathogenesis.—The bacillus is pathogenic for the domestic animals, all mammals seeming to be more or less sensitive to it. Birds are often immune. White mice are killed in five days, guinea-pigs in from eight to twelve days, rabbits in from four to five days, by virulent cultures. The morbid changes present include splenic tumor, hypertrophy of the thymus, and adenitis. In the rabbit there are, in addition, nephritis, enteritis, albuminuria, hemoglobinuria, and hemorrhages into the body-cavities.

The *dog is the most susceptible animal.* When it is injected intravenously the disease process almost immediately appears with such violent symptoms and such complex lesions as to recall the clinical and anatomic pictures of yellow fever in man. The most prominent symptom in the dog is vomiting, which begins directly after the penetration of the virus into the blood, and continues for a long time. Hemorrhages appear after the vomiting, the urine is scanty and albuminous, or is suppressed shortly before death. Grave jaundice was once observed.

Lesions.—The *post-mortem* lesions are highly interesting, and closely resemble those of yellow fever in man. Most conspicuous among them is profound steatosis of the liver. The liver-cells, even when examined fresh, appear completely degenerated into fat, this appearance corresponding to that found in fatal cases of yellow fever. The same result may be obtained by injecting the liver directly or through the abdominal wall. The kidneys are the seat of acute parenchymatous nephritis, sometimes with marked fatty degeneration. The whole digestive tract is the seat of hemorrhagic gastro-enteritis comparable in intensity only to poisoning by cyanid of potassium.

Experiments upon monkeys were also of interest, in-

asmuch as they demonstrate the possibility of obtaining fatty degeneration more extensive than is observed in man. In one case the liver was transformed into a mass of fatty substance similar to wax.

Goats and sheep are also very sensitive to the icteroid virus, and the lesions described also occur in them.

The death of a yellow fever victim is thought by Sanarelli to be the result of one of three causes:

1. It may be due to the specific infection principally, when Bacillus icteroides is found in the cadaver in a certain quantity and in a state of relative purity.
2. It may be due to the septicemias established during the course of the disease, the cadaver then presenting an almost pure culture of the other microbes.
3. It may be due in large measure to renal insufficiency, when the cadaver is found nearly sterile.

The black vomit is due to the action of gastric acidity upon the blood which has extravasated in the stomach in consequence of the toxic products of Bacillus icteroides.

Bacillus icteroides is said to produce a toxin the result of whose action corresponds to the essential symptoms of yellow fever. Animals immune against the infection, or only partially susceptible to it, are not much affected by the toxin. Susceptible animals, such as dogs, are profoundly affected. In from ten to fifteen minutes after injecting the toxin the animals experience a general rigor and abundant lachrymation, followed by continued vomiting, first of food, then of mucus. In a short time they lie helpless and extended. Hematuria frequently occurs. If the dose be moderate, recovery quickly follows the violent attack; but if the quantity of toxin be very large or repeated on successive days, it finally succumbs, presenting the anatomic lesions already described.

To prove the specificity of Bacillus icteroides, Sanarelli adduces five experimental inoculations upon men. These were not made with the bacteria,—*i. e.*, were not *infection experiments*,—but were made with the *filtered sterile toxin*, whose action could be controlled.

"The injection of the filtered cultures in relatively small doses reproduced in man typical yellow fever, accompanied by all its imposing anatomic and symptomatologic retinue—the fever, congestions, hemorrhages, vomiting, steatosis of the liver, cephalalgia, collapse—in short, all that complex of symptomatic and anatomic elements which in their combination constitute the indivisible basis of the diagnosis of yellow fever. This fact is not only striking evidence in favor

of the specific nature of the Bacillus icteroides, but it places the etiologic and pathologic conception of yellow fever on an altogether new basis."

The discovery of Bacillus icteroides, and especially of its toxin, entirely changes our view of the pathology of the disease. Instead of being a disease of the gastro-intestinal tract, as one would conclude from the symptoms, "all the symptomatic phenomena, all the functional alterations, all the anatomic lesions of yellow fever, are only the consequence of an eminently steatogenous, emetic, and hemolytic action of the toxic substances manufactured by Bacillus icteroides."

Wasdin and Geddings* confirmed in all points the work of Sanarelli, and believed Bacillus icteroides to be the specific cause of yellow fever. Archinard, Woodson, and Archinard† found that the blood of yellow fever cases caused agglutination with Bacillus icteroides and no other organism.

Agramonte ‡ refused to accept Bacillus icteroides as the cause of yellow fever, as he found it in only 7 out of 23 cases of yellow fever studied, and found it in other than yellow fever cadavers.

Readers interested in the study of yellow fever and the relationship of Bacillus icteroides to the disease should not fail to read the critical papers upon the subject by Novy.§

In a lengthy and interesting review and comparison of Sanarelli's and his own work, Sternberg ‖ concludes that Bacillus icteroides of Sanarelli is identical with Bacillus *x*, which he had discovered in yellow fever cadavers as early as 1888.

In a later paper** Sanarelli discusses the validity of Sternberg's claim to priority of discovery, and points out a sufficient number of differences in the original descriptions of the organisms to establish conclusively the individuality of Bacillus icteroides.

Bacillus of Havelburg.—About the same time that Sanarelli published his work, Havelburg †† announced the discovery of an entirely different bacillus, that he supposed to be specific for the disease. Without entering into a detailed description of Havelburg's bacillus, which seems to be far

* "Report of the Commission of Medical Officers Detailed by Authority of the President to Investigate the Cause of Yellow Fever," Washington, D. C., 1899.

† "New York Med. Jour.," Jan. 28, 1899.

‡ "Medical News," Feb. 10, 1900, vol. LXXVI, No. 6.

§ "Medical News," 1898.

‖ "Centralbl. f. Bakt. u. Parasitenk.," Sept. 6, 1897, Bd. XXII, Nos. 6 and 7.

** *Ibid.*, Bd. XXII, Nos. 22 and 23, p. 668.

†† "Ann. de l'Inst. Pasteur," 1897.

from established in importance, it may be classified as an interesting member of the colon group of bacilli.

It would seem, from a careful consideration of the recent literature, that Havelburg had very little ground for considering his bacillus specific, that it is scarcely possible for Sternberg to establish the identity of Bacillus *x* with Bacillus icteroides, and that none of these organisms are at all likely to be of etiological importance in yellow fever.

MOSQUITOS AND YELLOW FEVER.

Reed, Carroll, Agramonte,* and Lazear, constituting a Board of Medical Officers "for the purpose of pursuing scientific investigations with reference to the acute infectious diseases prevalent on the island of Cuba," began their work in 1900, at Havana, by a careful investigation of the relationship of Bacillus icteroides to yellow fever. By a most careful technic they withdrew and examined the blood from the veins of the elbow of 18 cases of yellow fever, making 48 separate examinations on different days of the disease, and preparing 115 bouillon cultures and 18 agar plates, every examination being negative so far as Bacillus icteroides was concerned. They were entirely unable to confirm the findings of Wasdin and Geddings, that Bacillus icteroides was present in blood obtained from the ear in 13 out of 14 cases, and concluded that both Sanarelli and Wasdin and Geddings were mistaken in their deductions.

In lieu of the remarkably interesting discoveries of Ronald Ross concerning the relation of the mosquito to malarial infection, the commissioners, remembering the theory of Finlay,† who published in 1881 an experimental research showing that mosquitos spread the infection of yellow fever, and the interesting and valuable observations of Carter‡ upon the interval between infecting and secondary cases of yellow fever, turned their attention to the mosquito. Securing mosquitos from Finlay and continuing the work where he had left it, they found that when mosquitos (*Stegomyia fasciata*) were permitted to bite patients suffering from yellow fever, after an interval of about twelve days they became able to impart yellow fever with their bites. This infectious character of the bite, having once de-

* "Phila. Med. Jour.," Oct. 27, 1900.

† "Annales de la Real Academia," vol. XVIII, 1881, pp. 147–169.

‡ "New Orleans Med. Jour.," May, 1900.

veloped, seems to remain throughout the subsequent life of the insect. So far as it was possible to determine, only one species of mosquito, Stegomyia fasciata, served as a host for the parasite whose cycles of development in the mosquito and in man must explain the symptomatology of yellow fever.

In order to establish these observations, experimental inoculations were made upon human beings in sufficient number to prove their accuracy. Unfortunately, Dr. Lazear, one of the victims of the experiment, lost his life from an attack of yellow fever induced by mosquito bites.

Major W. C. Gorgas * based the quarantine of yellow fever for the city of Havana upon the mosquito theory, and has had the extraordinary success of actually stamping out the disease. The quarantine and prophylaxis are very simple. The breeding-places of the stegomyia are drained and closed, and oil is poured upon unavoidable pools to prevent the embryo mosquitos from breathing. Yellow fever patients are protected from mosquitos, that might become infected, by nets, and non-immune persons are to sleep under mosquito canopies in rooms with screened windows.

Reed, Carroll, and Agramonte† came to the following conclusions:

1. The mosquito *C. fasciatus* serves as the intermediate host of yellow fever parasite.

2. Yellow fever is transmitted to the non-immune individual by means of the bite of the mosquito that has previously fed on the blood of those sick with the disease.

3. An interval of about twelve days or more after contamination appears to be necessary before the mosquito is capable of conveying the infection.

4. The bite of the mosquito at an earlier period after contamination does not appear to confer any immunity against a subsequent attack.

5. Yellow fever can be experimentally produced by the subcutaneous injection of blood taken from the general circulation during the first and second days of the disease.

6. An attack of yellow fever produced by the bite of a mosquito confers immunity against the subsequent injection

* International Sanitary Congress held at Havana, Cuba, Feb. 16 1902; Sanitary Department, Havana, series 4.

† Pan-American Medical Congress, Havana, Cuba, Feb. 4–7, 1901; Sanitary Department, Cuba, series 3, 1902.

of the blood of an individual suffering from the non-experimental form of the disease.

7. The period of incubation in thirteen cases of experimental yellow fever has varied from forty-one hours to five days and seventeen hours.

8. Yellow fever is not conveyed by fomites, and hence disinfection of articles of clothing, bedding, or merchandise, supposedly contaminated by contact with those sick with the disease, is unnecessary.

9. A house may be said to be infected with yellow fever only when there are present within its walls contaminated mosquitos capable of conveying the parasite of this disease.

10. The spread of yellow fever can be most effectually controlled by measures directed to the destruction of mosquitos and the protection of the sick against the bites of these insects.

11. While the mode of propagation of yellow fever has now been definitely determined, the specific cause of the disease remains to be discovered.

The probability that Bacillus icteroides is the specific cause and is transmitted by the mosquito is so slight that it need scarcely be considered. All analogy points to the organism being an animal parasite similar to that of malarial fever.

Concerning the prophylaxis of yellow fever, Guiteras * has studied the effect of intentionally permitting non-immunes who are to be exposed to the disease, to be experimentally infected by being bitten by infected mosquitos, after which they are at once carefully treated. His first conclusion was that "the intentional inoculation gives the patient a better chance of recovery," but the danger of death from the experimental injection was later shown to be so great that it had to be abandoned.

* "Revista de Medicina Tropical," Havana, Cuba, 1902.

CHAPTER V.

CHICKEN-CHOLERA.

Bacillus Choleræ Gallinarum (Perroncito).

General Characteristics.—A non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aerobic bacillus pathogenic for birds and mammals, staining by the ordinary methods, but not by Gram's method, producing acids, indol, and phenol, and coagulating milk.

The barnyards of both Europe and America are occasionally visited by an epidemic disease known as chicken-cholera, *Hühnercholera*, or *cholera de poule*, which rapidly destroys pigeons, turkeys, chickens, ducks, and geese. Rabbit-warrens are also at times affected and the rabbits killed.

The bacillus responsible for this disease was first observed by Perroncito * in 1878, and afterward thoroughly studied by Toussaint and Pasteur.†

Morphology.—The organisms are short and broad, with rounded ends, measuring 1×0.4–$0.6\ \mu$, sometimes joined to produce chains. Pasteur at first regarded them as diplococci, because the poles stain intensely, a narrow space between them remaining almost uncolored. This peculiarity is very marked, and careful examination is required to detect the intermediate substance. The bacillus does not form spores, is not motile, and has no flagella.‡

Staining.—The organism stains with ordinary anilin dye solutions, but not by Gram's method.

Cultivation.—Colonies.—Colonies upon gelatin plates appear after about two days as small, irregular, white points. The deep colonies reach the surface slowly, and do not attain any considerable size. The gelatin is not liquefied. The colonies appear under the microscope as irregularly rounded yellowish-brown disks with distinct smooth borders and

* "Archiv f. wissenschaftliche und praktische Thierheilkunde," 1879.

† "Compte-rendu de l'Acad. de Sci. de Paris," vol. xc.

‡ Thoinot and Masselin assert that the organism is motile. "Precis de Microbie," 2d ed., 1893.

granular contents. Sometimes there is a distinct concentric arrangement of the substance composing them.

Gelatin.—In gelatin puncture cultures a delicate white line occurs along the entire path of the wire. When viewed through a lens, this line is seen to consist of aggregated minute colonies. Upon the surface the development is much more marked, so that the growth resembles a nail with a pretty good sized flat head. If the bacilli be

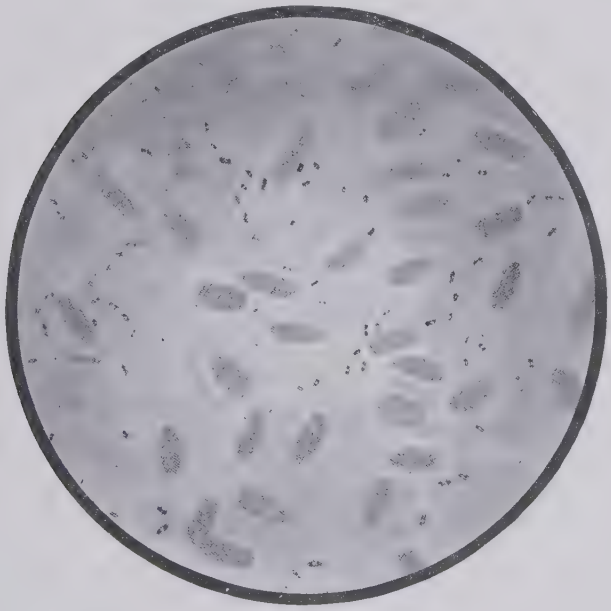


Fig. 156.—Bacillus of chicken-cholera, from the heart's blood of a pigeon. × 1000 (Fränkel and Pfeiffer).

planted upon the surface of obliquely solidified gelatin, a much more pronounced growth takes place, and along the line of inoculation a dry, granular coating is formed.

Bouillon.—The growth in bouillon is accompanied by a slight cloudiness.

Agar.—This growth, like that upon agar-agar and blood-serum, is white, shining, rather luxuriant, and devoid of characteristics.

Potato.—Upon potato no growth occurs except at the

incubation temperature. It is a very insignificant, yellowish-gray, translucent film.

Milk.—Milk is acidulated and slowly coagulated.

Vital Resistance.—The bacillus readily succumbs to the action of heat and dryness. The organism is an obligatory aerobe.

Metabolic Products.—Indol and phenol are formed by the organism.

Pathogenesis.—The introduction of cultures of this bacillus into chickens, geese, pigeons, sparrows, mice, and rabbits is sufficient to produce fatal septicemia. Feeding chickens, pigeons, and rabbits with material infected with the bacillus is also sufficient to produce the disease. Guinea-pigs, cats, and dogs seem immune, though they may succumb to large doses if given intraperitoneally. The organism is probably harmless to man.

Fowls ill with the disease fall into a condition of weakness and apathy, which causes them to remain quiet, seemingly almost paralyzed, and the feathers ruffled up. The eyes are closed shortly after the illness begins, and the birds gradually fall into a stupor, from which they do not awaken. The disease is fatal in from twenty-four to forty-eight hours. During its course there is profuse diarrhea, with very frequent fluid, slimy, grayish-white discharges.

Lesions.—The autopsy shows that when the bacilli are introduced subcutaneously a true septicemia results, with the formation of a hemorrhagic exudate and gelatinous infiltration at the seat of inoculation. The liver and spleen are enlarged; circumscribed, hemorrhagic, and infiltrated areas occur in the lungs; the intestines show an intense inflammation with red and swollen mucosa, and occasional ulcers following small hemorrhages. Pericarditis is frequent. The bacilli are found in all the organs. If, on the other hand, the disease has been produced by feeding, the bacilli are chiefly to be found in the intestine. Pasteur found that when pigeons were inoculated into the pectoral muscles, if death did not come on rapidly, portions of the muscle (*sequestra*) underwent degeneration and appeared anemic, indurated, and of a yellowish color.

Immunity.—Pasteur discovered that when cultures are allowed to remain undisturbed for several months, their virulence becomes greatly lessened, and new cultures transplanted from them are also attenuated. If chickens be

inoculated with such attenuated cultures, no other change occurs than a local inflammatory reaction that soon disappears and leaves the birds protected against future infection with virulent bacilli. From these observations Pasteur worked out a system of protective vaccination in which the fowls are first inoculated with attenuated, then with more active, and finally with virulent cultures, with resulting protection and immunity.

Use has been made of this bacillus to kill rabbits in Australia, where they are pests. It is estimated that two gallons of bouillon culture will destroy 20,000 rabbits irrespective of infection by contagion.

The bacillus of chicken-cholera may be identical with organisms found in various epidemic diseases of larger animals, and, indeed, no little confusion has arisen from the description of what is now pretty generally accepted to be the same organism as the bacillus of rabbit-septicemia (Koch), Bacillus cuniculicida (Flügge), bacillus of swine-plague (Löffler and Schütz), bacillus of "Wildseuche" (Hüppe), bacillus of "Büffelseuche" (Oriste-Armanni), etc.

CHAPTER VI.

HOG-CHOLERA.

Bacillus Suipestifer (Salmon and Smith).

General Characteristics.—An actively motile, flagellated, non-sporogenous, non-chromogenic, non-liquefying, aerobic and optionally anaerobic, aerogenic bacillus pathogenic for hogs and other animals. It stains by the ordinary methods, but not by Gram's method. It ferments dextrose, lactose, and sucrose, but does not form indol or coagulate or acidulate milk.

Hog-cholera, or "pig typhoid," as the English call it, is a common epidemic disease of swine, which at times kills 90 per cent. of the infected animals, and thus causes immense losses to breeders. Salmon estimates that the annual losses from this disease in the United States range from $10,000,000 to $25,000,000.

The bacillus of hog-cholera was first found by Salmon and Smith,* but was for a long time confused with the bacillus of "swine-plague," which it closely resembles and in association with which it frequently occurs. It is a member of the group of bacteria to which Bacillus icteroides and Bacillus typhi murium belong.

The organism was secured by Smith from the spleens of more than 500 hogs. It occurs in the blood and in all the organs, and has also been cultivated from the urine. Although so uniformly present in the disease and formerly accepted as its cause, the recent discovery that the serum of infected animals retains its infectivity after filtration through porcelain and the undoubted removal of any bacilli that it may formerly have contained, makes it very doubtful whether the so-called hog-cholera bacilli are more than accidental factors in the etiology of the disease.

Morphology.—The organisms appear as short rods with rounded ends, 1.2–1.5 μ long and 0.6–0.7 μ in breadth.

*"Reports of the Bureau of Animal Industry," 1885–91; and "Centralbl. f. Bakt. u. Parasitenk.," Bd. IX, Nos. 8, 9, and 10, March 2, 1891.

They are actively motile and possess long flagella (peritricha), easily demonstrable by the usual methods of staining. No spore-production has been observed. In general the bacillus resembles that of typhoid fever. It stains readily by the ordinary methods, but not by Gram's method.

Cultivation.—No trouble is experienced in cultivating

Fig. 157.—Ulceration of the intestine in a typical case of swine-fever (Crookshank).

the bacilli, which grow well in all the media under aerobic and anaerobic conditions.

Colonies.—Upon gelatin plates the colonies become visible in from twenty-four to forty-eight hours, the deeper ones appearing spheric with sharply defined borders. The

surfaces are brown by reflected light, and without markings. They are rarely larger than 0.5 mm. in diameter and are homogeneous throughout. The superficial colonies have little tendency to spread upon the gelatin. Their borders may be circular and rounded, or irregular. They rarely reach a greater diameter than 2 mm. The gelatin is not liquefied. There is nothing distinctly characteristic about them.

Upon agar-agar they attain a diameter of 4 mm. and have a gray, translucent appearance with polished surface. They are round and slightly arched.

Gelatin.—In gelatin punctures the growth takes the form of a nail with a flat head. There is nothing characteristic about it.

Agar-agar.—Linear cultures upon agar-agar present a translucent, circumscribed, grayish, smeary layer without characteristic appearances.

Potato.—Upon potato a yellowish coating is formed, especially when the culture is kept in the thermostat.

Bouillon.—Bouillon made with or without peptone is clouded in twenty-four hours. When the culture is allowed to stand for a couple of weeks without being disturbed, a thin surface growth can be observed.

Milk.—Milk is an excellent culture medium, but is not visibly changed by the growth of these bacteria. Its reaction remains alkaline.

Vital Resistance.—The bacillus is hardy. Smith found it vital after being kept dry for four months. It ordinarily dies sooner, however, and I have experienced difficulty in keeping it in the laboratory for any length of time unless frequently transplanted. The thermal death-point is 54° C., maintained for sixty minutes.

Metabolic Products.—Gas-production.—The hog-cholera bacillus is a copious gas-producer, capable of breaking up dextrose and lactose into CO_2, H, and an acid, which, formed late, eventually checks its further development. It does not ferment saccharose.

Indol.—No indol and no phenol are formed in the culture media.

Toxin.—In pure cultures of the hog-cholera bacillus Novy* found a poisonous base with the probable composition $C_{16}H_{26}N_2$, which he gave the provisional name "susotoxin."

* "Medical News," 1890, p. 231.

In doses of 100 mg. the hydrochlorid of this base causes convulsive tremors and death within one and one-half hours in white rats. He has also obtained a poisonous proteid of which 50 mg. were fatal for white rats, and which immunized them against highly virulent hog-cholera organisms when administered by repeated subcutaneous injection.

De Schweinitz* has also separated a slightly poisonous base which he calls "sucholotoxin," and a poisonous proteid that crystallizes in white, translucent plates when dried over sulphuric acid *in vacuo*, forms needle-like crystals with platinic chlorid, and was classed among the albumoses.

Pathogenesis.—The disease is particularly fatal to young pigs. The symptoms are not very characteristic, and the animals often die suddenly without having appeared very ill, or after seeming ill but a few hours. The chief symptoms consist of fever (106°–107° F.), unwillingness to move about, and more or less loss of appetite. The animals may appear stupid and dull, and have a tendency to hide in the bedding and remain covered by it. The bowels may be normal or constipated at the beginning of the attack, but later there is a liquid and fetid diarrhea, abundant, exhausting, and persisting to the end. The eyes are congested and watery, the secretion drying and gluing the lids together. The breathing is rapid, and there may be cough. Occasionally one sees an eruption with crusts or scabs of various sizes on the skin, which is often congested. The animal becomes weak, stands with arched back and drawn abdomen, and walks with a weak, tottering gait.

The course of this disease varies from one or two days to two or three weeks.

At post-mortem examination petechiæ, ecchymoses, and extravasations of blood into the tissues are found to be common and characteristic of the acute form of the disease. The spleen is enlarged to several times its normal size, and is soft and engorged with blood.

The extravasations of blood are common in the lymphatic glands, beneath the serous membranes of the thorax and abdomen, and particularly along the intestines; on the surface of the lungs and kidneys and in their substance. The contents of the intestine are sometimes covered with clotted blood. In the subacute form of the disease the principal changes are found in the large intestine, and consist of ulcers

* "Medical News," 1890, p. 237.

which appear as circular, slightly projecting masses varying in color from yellow to black. Occasionally these ulcers are slightly depressed. When cut across they are found to consist of a firm, solid growth extending nearly through the intestinal wall. They are most frequent in the cecum, upper half of the colon, and on the ileocecal valve. In the chronic form of the disease the spleen is rarely enlarged.

In hog-cholera the first effect of the disease is believed to be upon the intestines, with secondary invasion of the lungs.

The most characteristic lesions of the disease are the petechiæ and ecchymoses, the ulcerations of the large intestine (Fig. 157), the collapse, and the occasional broncho-pneumonic changes in the lung. Pulmonary changes, however, are more characteristic of swine-plague (*q. v.*).

The kidneys are nearly always affected, containing

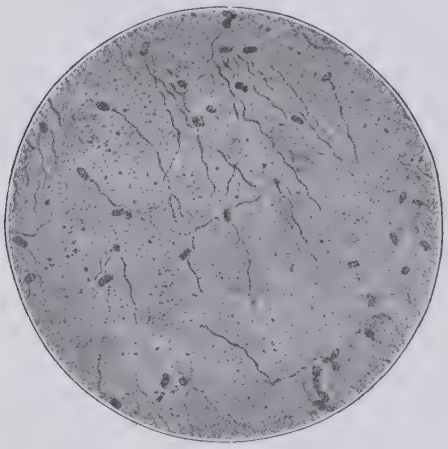


Fig. 158.—Bacillus of hog-cholera, showing flagella.

numerous petechial hemorrhages, and the urine containing albumin and tube-casts.

The bacillus is disappointing in its effects upon hogs. When they are subcutaneously or intravenously introduced or fed to such animals they sometimes fail to become diseased, sometimes show fever and depression, but rarely die. Animals thus made ill do not communicate the disease to others.

It seems, therefore, as if there was a radical difference between the natural and experimental hog cholera, and much doubt is thrown upon the specificity of the micro-organism. In the recent work of de Schweinitz and Dorset,* Niles† and Dorset, Bolton and McBryde,‡ it has been shown that there is a form of hog-cholera that can be transmitted by subcutaneous injection of the filtered and bacteria-free blood-serum of hogs ill of the disease, and that immunity could be conferred upon hogs by treatment with the desiccated blood and serum of such animals. This seems to make it certain that hog cholera is caused by some unseen and unknown virus and that the bacillus of Salmon and Smith is an incidental and probably accidental factor, not responsible for the disease itself, but only for certain of its clinical peculiarities. The form of hog-cholera resulting from successful inoculation with the bacillus is unlike the real disease in not being contagious, but that caused by the filtered blood-serum of sick hogs corresponds perfectly with the spontaneous disease, and is quite as contagious. The hog-cholera bacillus is markedly pathogenic for laboratory animals. Small quantities introduced subcutaneously into rabbits or mice kill them in from seven to twelve days. Guinea-pigs are less susceptible, 0.1 c.c. of a virulent culture often being required to kill them. The animal appears quite well for three or four days, then begins to sit quietly in the cage and eat but little, or refuses to eat at all, until death takes place.

Pigeons are still more refractory, and Smith found that 0.75 c.c. of a bouillon culture injected into the breast-muscles was required to kill them.

In Smith's experiments one four-millionth of a cubic centimeter of a bouillon culture injected subcutaneously into a rabbit was sufficient to cause its death. The temperature abruptly rises 2°–3° C., and remains high until death. Subcutaneous injection of larger quantities may kill in five days. Injected intravenously in small doses the bacillus may kill rabbits in forty-eight hours.

Lesions.—When the rabbit is examined post-mortem,

*Circular No. 41 of the Bureau of Animal Industry of the U. S. Dept. of Agriculture, 1903.

† Circular No. 43.

‡ Bull. No. 72, Bureau of Animal Industry, U. S. Dept. of Agriculture, 1905.

the spleen is found enlarged, firm, and dark red in color. The liver contains small yellowish-white necrotic areas which sometimes occur in one, sometimes in several acini, and not infrequently surround the interlobular veins. The kidneys are acutely inflamed and the urine is albuminous. The heart-muscle is spotted, gray, and fatty. In the intestinal tract the picture of the disease will be found to vary according to its duration. The contents of the small intestine are yellowish, watery, and mucous; Peyer's glands are enlarged. In the neighborhood of the pylorus, ecchymoses and extensive extravasations of blood are common. The bacilli are found in all of the organs.

In spite of the fact that hog-cholera is a disease of swine, and that it is from diseased and dead swine that the bacilli are obtained, these animals are not very easily infected artificially. They show no symptoms when injected subcutaneously, but almost invariably die after intravenous injection of 1–2 c.c. of a virulent culture.

Smith found that feeding with 200–300 c.c. of a bouillon culture after a day's fasting, or with small quantities administered daily, would cause death, with a widespread diphtheritic inflammation of the stomach and colon. Feeding with the organs of dead hogs produces the same lesions as the administration of the culture.

Immunity.—As early as 1886 Salmon and Smith found it possible to produce immunity against hog-cholera in susceptible animals, by gradually accustoming them to increasing doses of the bacteria. De Schweinitz isolated from cultures of the bacteria two toxic substances, a ptomain (sucholotoxin) and an albumose (sucholoalbumin), together with cadaverin and methylamin. With these substances he seems to have been able to produce immunity. Selander * and Metschnikoff found that immunity could be produced more quickly by the use of blood of infected rabbits exposed to 58° C. This blood was found to be exceedingly toxic.

De Schweinitz † found that the introduction into cows of progressively increased quantities of hog-cholera cultures caused the development of an antitoxic substance capable of protecting guinea-pigs from the disease.

After several years of treatment, some horses that I

* See "Centralbl. f. Bakt.," etc., Bd. XI, p. 339.

† "Centralbl. f. Bakt. u. Parasitenk.," XX, p. 573.

attempted to immunize failed to yield a serum protective enough to be of therapeutic value. To protect a rabbit against fatal infection required several cubic centimeters of the blood.

McClintock, Boxmeyer and Siffer* found that hogs may be immunized against hog-cholera by the simultaneous injection of diseased and immune blood, the larger animals with great readiness and apparently without danger, while the smaller animals react more violently and some loss is liable to occur. Drying at 37° C. or the addition of 33 per cent. glycerin does not attenuate the virus sufficiently for immunizing purposes. Moderate doses of immune serum show no curative power.

Agglutination.—Pitfield † found that after a single injection of a killed bouillon culture of the bacillus into a horse, the serum, which originally had very slight agglutinative power, showed a decided reaction. If the horse be immunized to large doses of such sterile cultures, the serum reaction becomes so marked that with a dilution of 1 : 10,000 a typical reaction occurs in sixty minutes.

McClintock, Boxmeyer and Siffer‡ found that the serum of normal hogs agglutinates strains of ordinary hog-cholera bacilli in dilution occasionally as high as 1 : 250 and consider reaction in a dilution of less than 1 : 300 without diagnostic value. The bacillus of swine dysentery is not agglutinated by normal blood in such high dilutions. The Widal reaction is of no value for the diagnosis of hog cholera as at present defined, but the presence of a positive reaction does, however, indicate an infection with hog-cholera bacilli. There are occasional instances of both natural and artificial infection in which no increase of the agglutinins for hog cholera over those normally present can be demonstrated. The minimal amount of agglutin develops in a hog's blood within six or seven days after a single inoculation with hog cholera vaccine. Hogs react to intraperitoneal injections of hog-cholera vaccine, usually with the production of large quantities of agglutins, the amount of the vaccine bearing no relation to the amount of agglutin produced.

* "Jour. Inf. Dis.," Mar. 1, 1905, vol. II, No. 2, p. 374

† "Microscopical Bulletin," 1897, p. 35.

‡ "Jour. of Infectious Diseases," vol. II, No. 2, p. 351, Mar. 1, 1905.

According to this experiment, in doubtful cases the use of this reaction should greatly facilitate the differentiation of the bacillus of hog-cholera from similar bacilli, and the serum test could be made use of to clinch the diagnosis of hog-cholera in doubtful cases of the disease in hogs.

CHAPTER VII.

SWINE-PLAGUE.

Bacillus Suisepticus (Löffler and Schütz).

General Characteristics.—A non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aerobic and optionally anaerobic bacillus, pathogenic for hogs and many other animals, staining by the ordinary methods, but not by Gram's method. It produces a slight acidity in milk, but does not coagulate it.

The bacillus of swine-plague, or Bacillus suisepticus of Löffler and Schütz * and Salmon and Smith,† but slightly resembles the bacillus of hog-cholera, though it was formerly

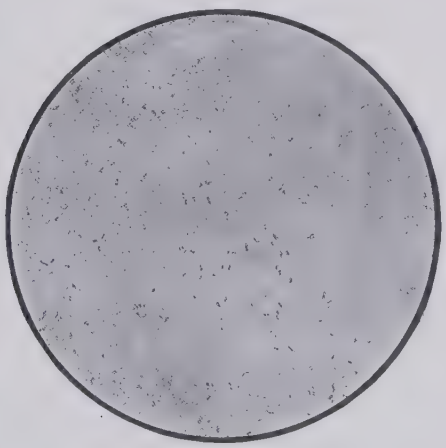


Fig. 159.—Bacillus of swine-plague (from photograph by E. A. de Schweinitz).

confounded with it and at one time thought to be identical. The species have sufficient well-marked characteristics, however, to make their differentiation easy (Fig. 159).

Swine-plague is a rather common and exceedingly fatal

* "Arbeiten aus dem kaiserlichen Gesundheitsamte," I.

† "Zeitschrift für Hygiene," X.

disease that not infrequently occurs in association with hog-cholera (*q. v.*), and because of the lack of sufficiently well-characterized symptoms—sick hogs appearing more or less alike—is often mistaken for it. The confusion resulting from such faulty diagnosis makes it difficult to determine exactly how fatal either disease may be in uncomplicated cases.

Morphology.—The bacillus of swine-plague much resembles that of hog-cholera, and not a little that of chicken-cholera. It is a short organism, rather more slender than the related species, not possessed of flagella, incapable of movement, and producing no spores.

In its growth the bacillus of swine-plague is an optional anaerobe.

Staining.—The bacillus stains by the ordinary methods, sometimes only at the poles, then closely resembling the bacillus of chicken-cholera. It is not colored by Gram's method.

Cultivation.—In general, the appearance in culture media is very similar to that of the hog-cholera bacillus. Kruse,* however, points out that when the bacillus grows in bouillon the liquid remains clear, the bacteria gathering to form a flocculent, stringy sediment. The organism does not grow upon ordinary acid potato, but if the reaction of the medium be alkaline, a grayish-yellow patch is formed. In milk a slight acidity is produced, but the milk is not coagulated.

Vital Resistance.—The vitality of the organism is low, and it is easily destroyed. Salmon says that it soon dies in water or when dried, and that the temperature for its growth must be more constant and every condition of life more favorable than for the hog-cholera germ. The organism is said to be widely distributed in nature, and is probably present in every herd of swine, though not pathogenic except when its virulence becomes increased or the vital resistance of the animals diminished by some unusual condition.

Pathogenesis.—While similar to hog-cholera, swine plague presents some marked differences, especially in regard to the seat of the local manifestations, to which attention has already been called, and in its duration, which is much shorter. There is also considerable resemblance to chicken-cholera, but the local reaction fol-

* Flügge's "Die Mikroorganismen," p. 419, 1896.

lowing the injection of the micro-organisms partakes of the nature of a hemorrhagic edema, which is not present in chicken-cholera, and rabbits especially commonly exhibit fatty metamorphosis of the liver.

Rabbits, mice, and small birds are very susceptible to the disease, usually dying of septicemia in twenty-four hours; guinea-pigs are less susceptible, except very young animals, which die without exception. Chickens are more immune, but usually succumb to large doses. Hogs die of septicemia after subcutaneous injection of the bacilli. There is a marked edema at the point of injection. If injected into the lung, a pleuro-pneumonia follows, with multiple necrotic areas in the lung. In these cases the spleen is not much swollen, there is slight gastro-intestinal catarrh, and the bacilli are present everywhere in the blood.

Animals can be infected only by subcutaneous, intravenous, and intraperitoneal inoculation, not by feeding.

As seen in hogs, the symptoms of swine-plague closely resemble those of hog-cholera, but differ in the existence of cough, swine-plague being prone to affect the lungs and oppress the breathing, which becomes frequent, labored, and painful, while hog-cholera is chiefly characterized by intestinal symptoms.

The course of the disease is usually rapid, and it may be fatal in a day or two.

Lesions.—At autopsy the lungs are found to be inflamed, and to contain numerous small, pale, necrotic areas, and sometimes large cheesy masses one or two inches in diameter. Inflammations of the serous membranes affecting the pleura, pericardium, and peritoneum, and associated with fibrinous inflammatory deposits on the surfaces, are common. There may be congestion of the mucous membrane of the intestines, particularly of the large intestine, or the disease in this region may be an intense croupous inflammation with the formation of a fibrinous exudative deposit on the surface.

A hemorrhagic form of the disease is said to be common in Europe, but, according to Salmon, is rare in the United States.

CHAPTER VIII.

TYPHUS MURIUM.

Bacillus Typhi Murium (Löffler).

General Characteristics.—A motile, flagellated, non-sporogenous, non-liquefying, non-chromogenic, aerobic and optionally anaerobic, aerogenic bacillus, pathogenic for mice and other small animals, staining by the ordinary methods, but not by Gram's method.

Bacillus typhi murium was discovered by Löffler* in 1889, when it created havoc among the mice in his laboratory at Greifswald.

Morphology.—The organism bears a close resemblance to that of typhoid fever, sometimes appearing short, sometimes long and flexible. There are many long and curly flagella with peritrichic arrangement, and the organism is actively motile. It does not produce spores.

Staining.—It stains with the ordinary dyes, but rather better with Löffler's alkaline methylene-blue.

Isolation.—The bacilli were first isolated from the blood of dead mice.

Cultivation.—Their cultivation presents no difficulties.

Colonies.—Upon gelatin plates the deep colonies are at first round, slightly granular, transparent, and grayish. Later they become yellowish-brown and granular. Superficial colonies are similar to those of the typhoid bacillus.

Gelatin.—In gelatin punctures there is no liquefaction. The growth takes place principally upon the surface, where a grayish-white mass slowly forms, and together with the growth in the puncture suggests a large flat-headed nail.

Agar-agar.—Upon agar-agar a grayish-white growth devoid of peculiarities occurs.

Potato.—Upon potato a rather thin whitish growth may be observed after a few days.

Milk.—The bacillus grows well in milk, causing acid reaction, without coagulation.

* "Centralbl. f. Bakt. u. Parasitenk.," xi, p. 129.

Pathogenesis.—The organism is pathogenic for mice of all kinds, which succumb in from one to two days when inoculated subcutaneously, and in from eight to twelve days when fed upon material containing the bacillus. The bacilli multiply rapidly in the blood- and lymph-channels, and cause death from septicemia.

Löffler expressed the opinion that this bacillus might be of use in ridding infested premises of mice, and its use for this purpose has been satisfactory in many places. He has succeeded in ridding fields so infested with mice as to be useless for agricultural purposes, by saturating bread with bouillon cultures of the bacillus and distributing it near their

Fig. 160.—Bacillus typhi murium (Migula).

holes. The bacilli not only killed the mice that had eaten the bread, but also infected others which ate their dead bodies, the extermination progressing until scarcely a mouse remained in the field.

In discussing the practical employment of this bacillus for the satisfactory destruction of field-mice, Brunner * calls attention to certain conditions that are requisite: (1) It is necessary, first of all, to attack extensive areas of the invaded territory, and not to attempt to destroy the mice of a small field into which an indefinite number of fresh animals may immediately come from surrounding fields. The

* "Centralbl. f. Bakt.," etc., Jan. 19, 1898, Bd. XXIII, No. 2, p. 68.

country-people, who are the sufferers, should combine their efforts so as to extend the benefits widely. (2) The preparation of the cultures is a matter of importance. Agar-agar cultures are most readily transportable. They are broken up in water, well stirred, and the liquid poured upon a large number of small pieces of broken bread. These are then distributed over the ground with care, being dropped into the fresh mouse-holes, and pushed sufficiently far in to escape the effects of sunlight upon the bacilli. Attention should be paid to holes in walls, under railway tracks, etc., and other places where mice live in greater freedom from disturbance than in the fields. (3) The destruction of the mice should be attempted only at a time of the year when their natural food is not plenty. By observing these precautions the mice can be eradicated in from eight to twelve days. In the course of two years no less than 250,000 cultures were distributed from the Bacteriological Laboratory of the Tierarznei Institut in Vienna, for the purpose of destroying field-mice.

The bacilli are not pathogenic for animals, such as the fox, weasel, ferret, etc., that feed upon the mice, do not affect man in any way, and so seem to occupy a useful place in agriculture by destroying the little but almost invincible enemies of the grain.

A somewhat similar organism, secured from an epidemic among field-mice and greatly increased in virulence by artificial manipulation, has been recommended by Danysz * for the destruction of rats. This organism, when subjected to a thorough study by Rosenau,† was found to be one of the paracolon bacilli, probably identical with Bacillus typhi murium of Löffler. It was too uncertain in action to accomplish satisfactorily the destruction of rats in plague-threatened cities for which it was suggested.

* "Ann. de l'Inst. Pasteur," April, 1900.

† Bulletin No. 5 of the Hygienic Laboratory of the U. S. Marine Hospital Service, Washington, D. C., 1901.

TABLE FOR THE DIFFERENTIATION OF CERTAIN BACTERIA RESEMBLING THE TYPHOID BACILLUS.

| Group. | Organism. | Discoverer. | Morphology. | | | | | | | Biologic Peculiarities. | | | | | | |

CHAPTER IX.

MOUSE-SEPTICEMIA.

Bacillus Rhusiopathiæ Suis (Kitt*)—Bacillus Muri-septicus (Koch).

General Characteristics.—An extremely minute, non-motile, non-flagellated, non-sporogenous, liquefying, aerobic and optionally anaerobic, pathogenic, non-chromogenic bacillus, staining by the ordinary methods and by Gram's method.

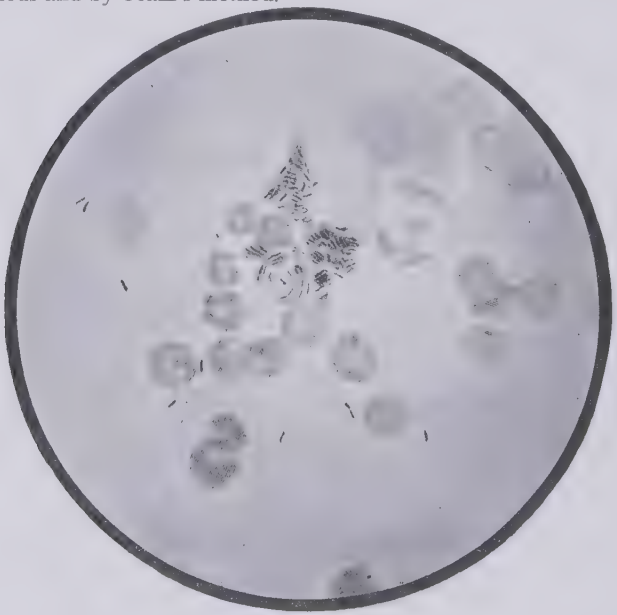


Fig. 161.—Bacillus of mouse-septicemia, from the blood of a mouse. × 1000 (Fränkel and Pfeiffer).

In 1878, during his investigations upon the infectious traumatic diseases, Koch † observed that when a minute quantity of putrid blood or of putrid meat-infusion was

* "Bakterienkunde und path. Mikroskopie," 1893, 284.

† "Wundinfektionskrankheiten," 1878.

injected into mice, the animals died of a septicemia caused by a minute bacillus to which he gave the name "Bacillus der Mäuseseptikämie" (Fig. 161).

In 1885 the bacillus was again brought into prominence by Löffler and Schütz, who found a supposedly identical organism in the erysipelatous disease (*rouget du porc*) affecting swine in many parts of Europe.

There seem to be certain slight morphologic and vegetative differences between these two organisms, but Baumgarten, Günther, Sternberg, and others have regarded them as insufficient for the creation of a separate species, and Lorenz has shown that immunity produced in the rabbit by a bacillus from the one source protects against a bacillus from the other.

Morphology.—The bacilli are extremely minute, measuring about 1.0 × 0.2 μ (Sternberg). Flügge, Fränkel, and Eisenberg find the bacillus of swine erysipelas somewhat shorter and stouter than that of mouse-septicemia.

Spores have been described by some observers, but it is very certain that none are ever formed.

Motility is ascribed by some (Schottelius and Fränkel) to Bacillus rhusiopathiæ suis, but neither organism is motile.

Fig. 162.—Colony of the bacillus of mouse-septicemia. ×80 (Flügge).

No flagella have been demonstrated.

Staining.—The bacilli stain well by the ordinary methods and by Gram's method.

Vital Resistance.—The organisms grow a little better without oxygen than with it. They are killed by a temperature of 52° C. in fifteen minutes, and die soon after drying.

Cultivation.—The organisms are easily cultivated from blood and tissues of infected animals and grow well in the ordinary media.

Colonies.—The colonies upon gelatin plates can first be seen on the second or third day, appearing as transparent grayish specks with irregular borders, from which many branched processes extend (Fig. 162). Fränkel describes them as resembling in shape the branched cells of the lacunæ of bone. When older, they coalesce and give the plate a cloudy gray appearance. The gelatin is not liquefied, but is gradually softened and its evaporation thus aided.

Gelatin Punctures.—In gelatin puncture cultures the growth is quite characteristic, and the tendency of the bacilli to grow anaerobically is well shown (Fig. 163). The development takes place all along the line of puncture, but is more marked below than at the surface. The growth occurs in a peculiar form, resembling superimposed disks, each disk separate from its neighbors and consisting of an area of clouded grayish gelatin reaching almost to the walls of the tube. This growth develops slowly, and causes a softening rather than an actual liquefaction of the gelatin.

Fig. 163.—Bacillus murisepticus (mouse-septicemia), gelatin stab culture, twelve days old (Curtis).

Agar-agar.—Upon agar-agar and blood-serum a very delicate, transparent grayish line develops along the path of the needle. It does not grow upon potato.

Vital Resistance.—The bacilli grow well at the room temperature, but much better at the temperature of the incubator (37° C.).

Pathogenesis.—The organisms are pathogenic for a variety of animals, notably hogs, rabbits, mice, white rats, pigeons, and sparrows. The guinea-pig, which is usually the victim of laboratory experiments, is immune. Field- and wood-mice, cattle, horses, asses, dogs, cats, chickens, and geese are also immune.

When mice are inoculated, they soon become ill, lose their appetites, mope in a corner, and are not readily disturbed. As the disease becomes worse they assume a sitting posture with the back much bent and the eyelids glued together by adhesive pus. When death comes, in the course of forty

to sixty hours after inoculation, they remain sitting in the same characteristic position.

When the ears of rabbits are inoculated, an inflammatory edema and distinct redness, much resembling erysipelas, occur. This lesion gradually spreads, involving the head and body of the animal, which ultimately dies.

When swine are affected, they become dull and weak, especially in the hind quarters; the temperature becomes elevated, and red patches appear upon the skin, which swells and becomes tender. Death follows in two or three days. Sixty per cent. of the diseased animals die.

Lesions.—In all animals the anatomic changes are much alike. The disease is a true bacteremia, and the bacilli can be found in the blood and in all the organs, especially the lungs and spleen. They are few in number in the streaming blood.

As the organisms stain well by Gram's method, this stain is of great value for their discovery in the tissues, and can be highly recommended.

The bacilli chiefly occupy the capillary blood-vessels, many of them being inclosed in leukocytes. The organs do not appear distinctly abnormal, except the spleen, which is enlarged. The mesenteric and other lymphatic nodes are also enlarged, and the gastric and intestinal mucous membranes are inflamed and mottled. The bacilli are also found in the intestinal contents, and Kitt, who discovered it, points out that the infection of swine probably takes place through the ingestion of the fecal matter of diseased animals.

Immunity.—Pasteur, Chamberland, Roux, and others have worked upon protective vaccinations based upon the attenuation of the virulence of the organism by passage through rabbits. Two vaccinations are said to be sufficient to produce immunity. The vaccinated animals, however, may be a source of infection to others, and should always be isolated. Klemperer in 1892 found that the blood-serum of immunized rabbits would save infected mice into which it was injected.

Lorenz in 1894 found an antitoxic substance in the blood of rabbits immunized against the disease. Its injection into other animals, however, affords only a temporary immunity. Later * he found it possible to protect hogs

* "Centralbl. f. Bakt. u. Parasitenk.," Jan., 1896, p. 168.

against the disease by injecting them with serum obtained from a hog immunized first with attenuated and then with virulent cultures of the bacillus. The strength of the serum can be determined by injecting it into mice infected with definite amounts of a culture of known virulence. The immunity thus produced lasted for a year.

CHAPTER X.

RELAPSING FEVER.

Spirochæta Obermeieri (Obermeier).

General Characteristics.—An elongate, flexible, flagellated, non-sporogenous, actively motile spiral bacterium, pathogenic for man and monkeys, not susceptible of cultivation in artificial media, stained by ordinary methods, but not by Gram's method.

In 1873 Obermeier* discovered a flexible spiral organism in the blood of patients suffering from relapsing fever, but in spite of careful study we have advanced but little in our knowledge of it. The recent researches of Schau-

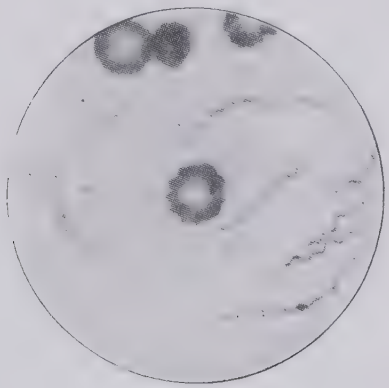


Fig. 164.—Spirochæta obermieri from human blood (Kolle and Wassermann).

dinn upon the trypanosomes and the discovery that certain ticks and probably also bedbugs act as definite hosts of the parasites have led to the supposition that this organism, instead of being a true spirochæta, may be a trypanosome. Its appearance and general structure are,

* "Centralbl. f. d. med. Wissenschaft," 1873.

however, against this. It is long, very slender, divides by transverse fission, possesses two terminal flagella, has no undulating membrane, and when most painstakingly stained does not display a major and minor nucleus or centrosome, such as is usually seen in trypanosomes. In favor of the trypanosome theory may be mentioned the occurrence of insects as hosts of the parasites and our inability to grow them upon artificial culture media.

The organism is undoubtedly the cause of relapsing fever. It can be found in the blood in all cases during the febrile paroxysms and is never found in any other affection. The introduction of it into the blood of monkeys produces relapsing fever in these animals, and recently it has been shown by Novy that the organism is usually infectious for white rats. An attack of the disease is followed by immunity of long duration, and the serum of rats that have been experimentally infected is protective for other rats.

CHAPTER XI.

BUBONIC PLAGUE.

Bacillus Pestis Bubonicæ (Yersin, Kitasato).

General Characteristics.—A minute, pleomorphous, diplococcoid and elongate, sometimes branched, non-motile, non-flagellated, non-sporogenous, non-chromogenic, aerobic and optionally anaerobic, pathogenic organism, specific for bubonic plague, easily cultivated artificially, and susceptible of staining by ordinary methods, but not by Gram's method.

Plague, bubonic plague, pest, or malignant polyadenitis is an acute infectious febrile disease of an intensely fatal nature, characterized by inflammatory enlargement and softening of the lymphatic glands, marked pulmonary, cerebral, and vascular disturbance, and the presence of the specific bacillus in the lymphatic glands and blood.

It is an extremely fatal affection, whose ravages in the hospital at Hongkong, in which Yersin made his original observations, carried off 95 per cent. of the cases. The death-rate varies in different epidemics from 50 to 90 per cent. In the epidemic at Hongkong in 1894 the death-rate was 93.4 per cent. for Chinese, 77 per cent. for Indians, 60 per cent. for Japanese, 100 per cent. for Eurasians, and 18.2 per cent. for Europeans. It affects both men and animals, and is characterized by sudden onset, high fever, prostration, delirium, and the occurrence of lymphatic swellings—buboes—affecting chiefly the inguinal glands, though not infrequently the axillary, and sometimes the cervical, glands. Death comes on in severe cases in forty-eight hours. The pneumonic form is most rapidly fatal. The longer the duration of the disease, the better the prognosis. Autopsy in fatal cases reveals the characteristic enlargement of the lymphatic glands, whose contents are soft and sometimes purulent.

Wyman,* in his very instructive pamphlet, "The Bubonic Plague," finds it convenient to divide plague into (*a*) bubonic or ganglionic, (*b*) septicemic, and (*c*) pneumonic

*Government Printing Office, Washington, D. C., 1900.

forms. Of these, the bubonic form is most frequent and the pneumonic form most fatal.

The infection usually takes place through some peripheral lesion, but may occur by inhalation of the specific organisms.

The bacillus of bubonic plague (Fig. 165) was independently discovered by Yersin * and Kitasato † in the summer of 1894, during an epidemic of the plague then raging at Hongkong. There seems to be little doubt but that the micro-organisms described by the two observers are identical.

Morphology.—The bacillus is quite variable. Usually it is short and thick,—a "cocco-bacillus," as some call it,—with rounded ends. Its size is small (1.5 to 2 μ in length)

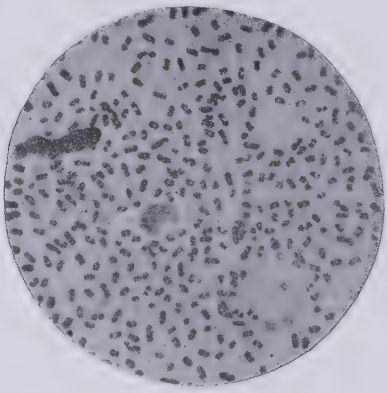


Fig. 165.—Bacillus of bubonic plague (Yersin).

and 0.5 to 0.75 in breadth. It not infrequently occurs in chains of four or six or even more, and is occasionally encapsulated. It shows active Brownian movements, which probably led Kitasato to consider it motile. Yersin did not regard it as motile, and was correct. Gordon‡ claims that some of the bacilli have flagella. No spores are formed.

Staining.—It stains by the usual methods; not by Gram's method. When stained, the organism rarely appears uniformly colored, being darker at the ends than

* "Ann. de l'Inst. Pasteur," 1894, 9.

† Preliminary notice of the bacillus of bubonic plague, Hongkong, July 7, 1894.

‡ "Centralbl. f. Bakt. u. Parasitenk.," Sept. 6, 1897, Bd. XXII, Nos. 6 and 7, p. 170.

at the center, so as to resemble a dumb-bell or diplococcus. The bacilli sometimes appears vacuolated, and nearly all cultures show a variety of involution forms. Kitasato has compared the general appearance of the bacillus to that of chicken-cholera.

Involution forms on partly desiccated agar-agar not containing glycerin are said by Haffkine to be characteristic. The microbes swell and form large, round, oval, pea- or spindle-shape or biscuit-like bodies which may attain twenty times the normal size and in growing gradually lose the ability to take up the stain. Such involution forms are not seen in liquid culture.

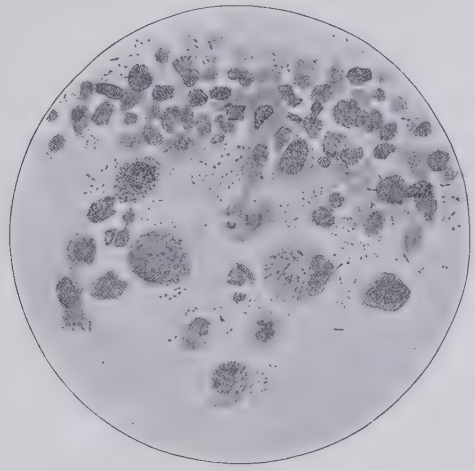


Fig. 166.—Bacilli of plague and phagocytes, from human lymphatic gland. × 800 (Aoyama).

Ogata * states that while Kitasato found the bacillus in the blood of cadavers, Yersin seldom found it in the blood, but always in the enlarged lymphatic glands; that Kitasato's bacillus retains the color when stained by Gram's method; Yersin's does not; that Kitasato's bacillus is motile, Yersin's non-motile; that the colonies of Kitasato's bacillus, when grown upon agar, are round, irregular, grayish-white with a bluish tint, and resemble glass-wool when slightly magnified; those of Yersin's bacillus, white and transparent,

* "Centralbl. f. Bakt. u. Parasitenk.," June 24, 1897, Bd. xxi, Nos. 20 and 21.

with iridescent edges. Ogata, in his investigations, found that the bacillus corresponded with the description of Yersin, rather than that of Kitasato, and it is certain that the description given by Yersin is the more correct of the two.

In the "Japan Times," Tokio, November 28, 1899, Kitasato explains that, his investigations being made upon cadavers that were partly putrefied, he was led to believe that the bacillus first invaded the blood. Later studies upon living subjects showed him the error of this view and the correctness of Yersin's observation that the bacilli first multiply in the lymphatics.

Both Kitasato and Yersin showed that in blood drawn from the finger-tips and in the softened contents of the glands the bacillus may be demonstrable.

Cultivation.—When cultures are made from the blood or softened contents of the buboes, the bacillus may be obtained in pure culture, and develops well upon artificial culture media.

Bouillon.—In bouillon, a diffuse cloudiness was observed by Kitasato, though Yersin observed that the cultures resembled erysipelas cocci, and contained zooglea attached to the sides and at the bottom of the tube of nearly clear fluid.

Haffkine* found that when an inoculated bouillon culture is allowed to stand perfectly at rest, on a firm shelf or table, a characteristic appearance develops. In from twenty-four to forty-eight hours, the liquid remaining limpid, flakes appear underneath the surface, forming little islands of growth, which in the next twenty-four to forty-eight hours grow into a jungle of long stalactite-like masses, the liquid remaining clear. In from four to six days these islands become still more compact. If the vessels be disturbed, they fall like snow and are deposited at the bottom, leaving the liquid clear.

Colonies.—Upon gelatin plates at 22° C. the colonies may be observed in twenty-four hours by the naked eye. They are pure white or yellowish-white, spheric when deep in the gelatin, flat when upon the surface, and are about the size of a pin's head. The gelatin is not liquefied. Upon microscopic examination the borders of the colonies are found to be sharply defined. The contents become more granular as

* "Brit. Med. Jour.," June 12, 1897, p. 1461.

the age increases. The superficial colonies are occasionally surrounded by a fine, semi-transparent zone.

Gelatin Punctures.—In gelatin puncture cultures the development is scant. The medium is not liquefied; the growth takes place in the form of a fine duct, little points being seen on the surface and in the line of puncture. Sometimes fine filaments project into the gelatin from the central puncture.

Agar-agar.—Upon agar-agar the bacilli grow freely, but slowly, the colonies being whitish in color, with a bluish tint by reflected light, and first appearing to the naked eye when cultivated from the blood of an infected animal after about thirty-six hours' incubation at 37° C. Under the microscope they appear moist, with rounded, uneven edges. The small colonies are said to resemble tufts of glass-wool. Microscopic examination of the agar-agar culture shows the presence of chains resembling streptococci.

Upon glycerin agar the development of the colonies is slower, though in the end the colonies attain a larger size than those grown upon plain agar.

Klein * says that the colonies develop quite readily upon gelatin made from beef bouillon (not infusion), appearing in twenty-four hours, at 20° C., as small, gray, irregularly rounded dots. Magnification shows the colonies to be serrated at the edges and made up of short, oval, sometimes double bacilli. Some colonies contrast markedly with their neighbors in that they are large, round, or oval, and consist of longer or shorter, straight or looped threads of bacilli. The appearance was much like that of the young colonies of Proteus vulgaris. At first these were regarded as contaminations, but later he was led to believe that their occurrence was characteristic of the plague bacillus. The peculiarities of these colonies cannot be recognized after forty-eight hours.

Hankin and Leumann † recommend for the differential diagnosis of the plague bacillus a culture medium prepared by the addition of 2.5–3.5 per cent. of salt to ordinary culture agar-agar. When transplanted from ordinary agar-agar to the salt agar-agar, the involution forms so charac-

* "Centralbl. f. Bakt. u. Parasitenk.," July 10, 1897, XXI, Nos. 24 and 25.

† "Centralbl. f. Bakt. u. Parasitenk.," Oct., 1897, Bd. XXII, Nos. 16 and 17, p. 438.

teristic of the bacillus are formed with exceptional rapidity. In bouillon containing this high percentage of salt the stalactite formation is beautiful and characteristic.

Blood-serum.—Upon blood-serum growth at the temperature of the incubator is luxuriant and forms a moist layer, of a yellowish-gray color, unaccompanied by liquefaction of the serum.

Potato.—Upon potato no growth occurs at ordinary temperatures. When the potato is stood in the incubator for a few days, a scanty, dry, whitish layer develops.

Abel found the best culture medium to be 2 per cent. alkaline peptone solution containing 1 or 2 per cent. of gelatin, as recommended by Yersin and Wilson.

Vital Resistance.—Kitasato found that the plague bacillus did not seem able to withstand desiccation longer than four days; but Rappaport * found that they remained alive when kept dry upon woolen threads at 20° C. for twenty-three days, and Yersin found that although it could be secured from the soil beneath an infected house at a depth of 4–5 cm., the virulence of such bacilli was lost.

Kitasato found that the bacillus was killed by two hours' exposure to 0.5 per cent. carbolic acid, and also by exposure to a temperature of 80° C. for five minutes. Ogata found the bacillus instantly killed by 5 per cent. carbolic acid, and in fifteen minutes by 0.5 per cent. carbolic acid. In 0.1 per cent. sublimate solution it is killed in five minutes.

According to Wyman, the bacillus is killed by exposure to 55° C. for ten minutes. The German Plague Commission found that the bacilli were killed by exposure to direct sunlight for three or four hours; and Bowhill † found that they are killed by drying at ordinary room temperatures in about four days.

Wilson ‡ found the thermal death-point of the organism one or two degrees higher than that of the majority of non-sporulating pathogenic bacteria, and that the influence of sunlight and desiccation cannot be relied upon to destroy it.

Rosenau § found temperature the most important factor,

* Quoted by Wyman.

† "Manual of Bacteriological Technique and Special Bacteriology," 1899, p. 197.

‡ "Journal of Medical Research," vol. VI, No. 1, p. 53, July, 1901.

§ Bulletin No. 4 of the Hygienic Laboratory of the U. S. Marine Hospital Service, 1901.

as it dies quickly when kept dry at 37° C., but remains alive for months when kept dry at 19° C. Sunlight kills it in a few hours. A temperature of 70° C. is invariably fatal in a short time.

Metabolism.—The bacillus develops under conditions of aerobiosis and anaerobiosis. In glucose-containing media it does not form gas. No indol is formed. Ordinarily the culture medium is acidified, the acid reaction persisting for three weeks or more.

Experimental Infection.—Mice, rats, guinea-pigs, rabbits, monkeys, dogs, and cats are all susceptible to experimental inoculation. During epidemics the purely herbivorous animals usually escape, though oxen have been known to die of the disease. When blood, lymphatic pulp, or pure cultures are inoculated into them, the animals become ill in from one to two days, according to their size and the virulence of the bacillus. Their eyes become watery, they show disinclination to take food or to make any bodily effort, the temperature rises to 41.5° C., they remain quiet in a corner of the cage, and die with convulsive symptoms in from two to seven days. If the inoculation be made intravenously, no lymphatic enlargement occurs; but if it be made subcutaneously, the nearest lymph-nodes always enlarge and suppurate if the animal live long enough. The bacilli are found everywhere in the blood, but not in very large numbers.

Rats seem to suffer from a chronic form of the disease, and sometimes can be found to have encapsulated caseous nodules in the submaxillary glands, caseous bronchial glands, and fibroid pneumonia months after inoculation. In all such cases virulent plague bacilli are present. This matter is important to the epidermiologist.

According to Yersin, an infiltration or watery edema can be observed in a few hours about the point of inoculation. The autopsy shows the infiltration to be made up of a yellowish gelatinous exudation. The spleen and liver are enlarged, the former often presenting an appearance similar to that observed in miliary tuberculosis. Sometimes there is universal enlargement of the lymphatic glands. Bacilli are found in the blood and in all the internal organs. Skin eruptions may occur during life, and upon the inner abdominal walls petechiæ and occasional hemorrhages may be found. The intestine is hyperemic, the adrenals congested.

Sero-sanguinolent effusions may occur into the serous cavities.

Devell * has found frogs susceptible to the disease.

Wyssokowitsch and Zabolotny † found monkeys highly susceptible to plague, especially when subcutaneously inoculated. When an inoculation was made with a pin dipped in a culture of the bacillus, the puncture being made in the palm of the hand or sole of the foot, the monkeys always died in from three to seven days. In these cases the local edema observed by Yersin did not occur. They point out the interest attaching to infection through so insignificant a wound and without local lesions.

Klein ‡ found that intraperitoneal injection of the bacillus into guinea-pigs was of diagnostic value, producing a thick, cloudy, peritoneal exudate rich in leukocytes and containing characteristic chains of the plague bacillus occurring in twenty-four to forty-eight hours.

Animals fed upon cultures of the bacillus or upon the flesh of animals dead of the disease became ill and died with typical symptoms. When Klein inoculated animals with the dust of dwelling-houses in which the disease had occurred, some of them died of tetanus, one from plague. Many rats and mice died spontaneously in Hongkong, examination showing the characteristic bacilli.

Yersin showed that flies may die of the disease. Macerating and crushing a fly in bouillon, he not only succeeded in obtaining the bacillus, but infected an animal with it.

Nuttall, § in repeating Yersin's fly experiment, found his observation correct, and showed that flies fed with the cadavers of plague-infected mice die in a variable length of time. Large numbers of plague bacilli were found in their intestines. He also found that bedbugs allowed to prey upon infected animals took up large numbers of the plague bacilli and retained them for a number of days. These bugs did not, however, infect healthy animals when allowed to bite them; but Nuttall was not satisfied that the number of his experiments upon this point was great enough to prove that plague cannot be spread by the bites of suctorial insects.

* "Centralbl. f. Bakt. u. Parasitenk.," Oct. 12, 1897.

† "Ann. de l'Inst. Pasteur," Aug. 25, 1897, XI, 8, p. 665.

‡ "Centralbl. f. Bakt. u. Parasitenk.," XXI, No. 24, July 10, 1897, p. 849.

§ *Ibid.*, Aug. 13, 1897.

Ogata found plague bacilli in fleas taken from diseased rats. He crushed some fleas between sterile object-glasses and introduced the juice into the subcutaneous tissues of a mouse, which died in three days with typical plague, a control animal remaining well. Some guinea-pigs taken for experimental purposes into a plague district died spontaneously of the disease, presumably because of insect infection.

The animal most prone to spontaneous infection seems to be the rat, and there is much evidence in support of the view that it aids in the spread of epidemics. In several of the Asiatic plague districts and at Santos the appearance of plague among the inhabitants was preceded by a large mortality among the rats, which examination showed had died of plague.

Galli-Valerio * and others think that the fleas of the mouse and rat are incapable of living upon man and do not bite him, and that it is only the *Pulex irritans*, or human flea, that could be capable of transmitting the disease from man to man. Tidswell,† however, found that of 100 fleas collected from rats, there were four species, of which three—the most common kinds—bit men as well as rats. Lisbon‡ found that of 246 fleas caught on men in the absence of plague, only one was a rat flea, but out of 30 fleas caught upon men in a lodging-house during plague 14 were rat fleas. This seems to show that as the rats die off, their fleas seek new hosts and may thus contribute to the spread of the disease.

It may be that the fleas, while not themselves making the inoculation, deposit the bacilli in their feces upon the skin, into which it is carried by the finger-nails when the bites are scratched. M. Herzog§ has shown that pediculi may harbor plague bacilli and act as carriers of the disease.

Diagnosis.—It seems possible to make a diagnosis of the disease in doubtful cases by examining the blood, but it is admitted that a good deal of bacteriologic practice is necessary for the purpose.

Abel found that blood-examinations may yield doubtful results because of the variable appearance of the contained bacilli, which may easily be mistaken for other bacteria. He deems the best tests to be the inoculation of

* "Centralbl. f. Bakt. u. Parasitenk.," Jan. 6, 1900, XXVII, No. 1, p. 1.

† "British Med. Journal," June 27, 1903.

‡ "Times of India," Nov. 26, 1904.

§ "Amer. Jour. Med. Sci.," Mar., 1905.

broth cultures and the subsequent inoculation into animals, which, he advises, should have been previously vaccinated against the streptococcus. Plague bacilli sometimes persist in the urine for a week after convalescence.

Kolle* has suggested a method valuable both for the diagnosis of the disease and for estimating the virulence of the bacillus. It is as follows: "The skin over a portion of the abdominal wall of the guinea-pig is shaved, care being taken to avoid the slightest injury of the skin. The infective material is carefully rubbed into the shaved skin. Important, in order rightly to understand the occurrence of plague infection, is the fact disclosed here in the case of guinea-pigs, that by this method of inoculation the animals present the picture of true bubonic plague—that is to say, the production of nodules in the various organs, principally in the spleen. In this manner guinea-pigs, which would not be affected by large subcutaneous injections, even amounting to 2 mg. of agar culture (equal to a loop) of low-virulence plague bacillus, may be infected and eventually succumb."

Virulence.—By frequent passage through animals of the same species the bacillus can be much increased in virulence. Kolle recommends rats for this purpose, and, indeed, declares that without the use of rats it is impossible to keep cultures at a high grade of virulence. Batzaroff found that the most virulent plague germs were to be obtained from the pneumonic lungs of rats that had been infected through the nasal aperture with cotton-wool saturated with a culture of the bacillus. This is not, however, a reliable method of inoculation.

Yersin found that when cultivated for any length of time upon culture media, especially agar-agar, the virulence was rapidly lost and the bacillus eventually died. On the other hand, when constantly inoculated from animal to animal, the virulence of the bacillus is much increased.

Knorr, Yersin, Calmette, and Borrel† have shown that the bacillus made virulent by frequent passage through mice is not increased in virulence for rabbits.

This no doubt depends upon the sensitivity of the bacillus to the protective substances of the body-juices, immuniza-

*See Havelburg, "Public Health Reports," Aug. 15, 1902, vol. XVII, No. 33, p. 1863.

†"Ann. de l'Inst. Pasteur," July, 1895.

tion against those of one animal not necessarily protecting the organism against those of other animals.

Bielonovsky * finds that broth, agar, and serum cultures of the plague bacillus possess the property of hemolyzing the blood of normal animals. The hemolytic power of filtrates of plague cultures increases up to the thirteenth or fourteenth day, then gradually diminishes, but without completely disappearing. The hemolysins are notably resistant to heat, not being destroyed below 100° C.

Immunity.—Kitasato's experiments first showed that it was possible to bring about immunity against the disease, and Yersin, working in India, and Fitzpatrick, in New York, have successfully immunized large animals (horses, sheep, and goats). The serum of the immunized animals contains an antitoxin capable not only of preventing the disease, but also of curing it in mice and guinea-pigs and probably in man.

Haffkine's Prophylactic.—Haffkine † followed his plan of preventive inoculation as employed against cholera, and has invented a mode of prophylaxis based upon the use of devitalized cultures. Bouillon cultures are used, and small floating drops of butter are employed to make the "islands" of plague bacilli float. The cultures are grown for a month or so, successive crops of the island-stalactite growth being precipitated by agitating the tube. In this manner an "intense extracellular toxin" containing large numbers of the bacilli is prepared. The culture was killed by exposure to 70° C. for one hour, and used in doses of 1–3 c.c. as a preventive inoculation.

An interesting collection of statistics, showing in a convincing manner the value of the Haffkine prophylactic, is published of Leumann, of Hubli. The figures, together with a great deal of interesting information upon the subject, can be found in the paper upon "A Visit to the Plague Districts in India," by Barker and Flint.‡

The immunity conferred by the Haffkine prophylactic lasts about a month. The preparation must never be used if the person has already been exposed to infection, and is in the incubation stage of the disease, as it contains the toxins of the disease, and therefore greatly intensifies the existing condition. When injected into healthy persons,

* "Arch. des Sci. Biol.," Tome x, No. 4, St. Petersb., 1904.

† "Brit. Med. Jour.," June 12, 1897.

‡ "New York Med. Jour.," Feb. 3, 1900.

it always produces some fever, slight local swellings, and malaise.

Wyssokowitsch and Zabolotny,* whose studies have already been quoted, used 96 monkeys in the study of the value of the "plague serums," and found that when treatment is begun within two days from the time of inoculation the animals can be saved, even though symptoms of the disease are marked. After the second day the treatment cannot be relied upon. The dose necessary was 20 c.c. of a serum having a potency of 1 : 10. If too little serum was given, the course of the disease was retarded and the animal improved for a time, then suffered a relapse, and died in from thirteen to seventeen days. The serum also produced immunity, but of only ten to fourteen days' duration. Immunity lasting three weeks was conferred by inoculating a monkey with an agar-agar culture heated to 60° C. If too large a dose of such a culture was given, however, the animal was enfeebled and remained susceptible.

Of Yersin's serum, which is prepared by immunizing horses against the toxins and cultures of the bacillus in the usual manner, 5 c.c. doses have been found to confer an immunity lasting for about a fortnight. Larger doses confer a longer immunity. For the treatment of the developed disease in man, doses of 50 and even 100–200 c.c. seem necessary to produce the desired effect.

* *Loc. cit.*

CHAPTER XII.

TETRAGENUS.

Micrococcus Tetragenus (Gaffky).

General Characteristics.—Large, round, encapsulated cocci, regularly associated in groups of four, forming tetrads. They are non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, non-aerogenic, aerobic and optionally anaerobic, pathogenic for mice and other small animals, and stain well by all methods, including that of Gram.

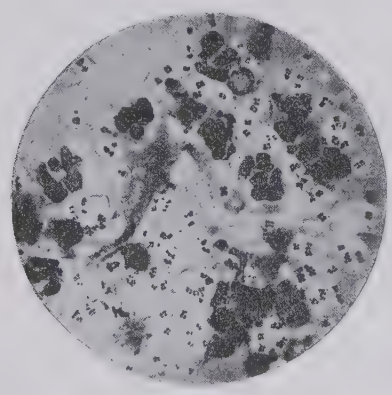


Fig. 167.—Micrococcus tetragenus in pus from a white mouse. × 615 (Heim).

A large micrococcus grouped in fours and known as Micrococcus tetragenus (Fig. 167) can sometimes be found in normal saliva, tuberculous sputum, and more commonly in the contents of the cavities of tuberculosis pulmonalis. It sometimes occurs in the pus of acute abscesses, and may be of importance in connection with the pulmonary abscesses which complicate tuberculosis. It was discovered by Gaffky.*

Morphology.—The cocci are rather large, measuring about 1 μ in diameter. In cultures they do not show the regu-

* "Archiv für Chirurgie," 28, 3.

lar arrangement in tetrads as constantly as in the blood and tissues of animals where they occur in groups of four surrounded by a transparent gelatinous capsule.

Staining.—The organisms stain well by ordinary methods, and beautifully by Gram's method, by which they can be best demonstrated in tissues.

Isolation.—The organism can be isolated by inoculating a white mouse with sputum or pus containing it. After death it can be recovered from the blood.

Cultivation.—It grows readily upon artificial media. Upon gelatin plates small white colonies are produced in from twenty-four to forty-eight hours. Under the microscope they appear spheric or elongate (lemon-shaped), finely

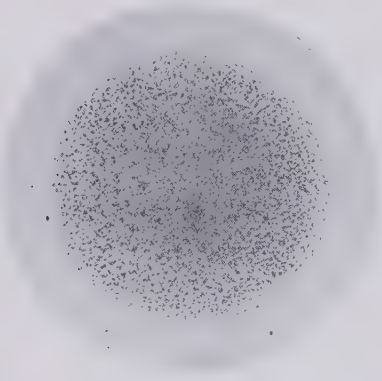


Fig. 168.—Micrococcus tetragenus; colony twenty-four hours old upon the surface of an agar-agar plate. × 100 (Heim).

granular, and lobulated like a raspberry or mulberry. When superficial they are white and elevated, 1–2 mm. in diameter (Fig. 168).

Gelatin.—In gelatin punctures a large white surface growth takes place, but development in the puncture is very scant, the small spheric colonies usually remaining isolated. The gelatin is not liquefied.

Agar-agar.—Upon agar-agar spheric white colonies are produced. They may remain discrete or become confluent.

Potato.—Upon potato a luxuriant, thick, white growth is formed.

Blood-serum.—The growth upon blood-serum is also

abundant, especially at the temperature of the incubator. It has no distinctive peculiarities.

Pathogenesis.—The introduction of tuberculous sputum or of a minute quantity of a pure culture of this coccus into white mice usually causes a fatal bacteremia.

The organisms are found in small numbers in the heart's blood, but are numerous in the spleen, lungs, liver, and kidneys.

House-mice and field-mice, dogs and rabbits are comparatively immune. Guinea-pigs die of general septic infection, though local abscesses result from subcutaneous inoculation.

The tetracocci, when present, probably hasten the tissue-necrosis in tuberculous cavities, aid in the formation of abscesses of the lung, and contribute to the production of the hectic fever.

An interesting contribution to the relationship of this coccus to human pathology has been made by Lartigau,* who succeeded in demonstrating that the tetracoccus may be the cause of a pseudo-membranous angina, three cases of which came under his observation.

* "Phila. Med. Jour.," April 22, 1899.

CHAPTER XIII.

INFLUENZA.

Bacillus Influenzæ (R. Pfeiffer).

General Characteristics.—A minute, non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aerobic, pathogenic bacillus, staining by the ordinary methods, but not by Gram's method, and susceptible of artificial cultivation, chiefly through the addition of hemoglobin to the culture media.

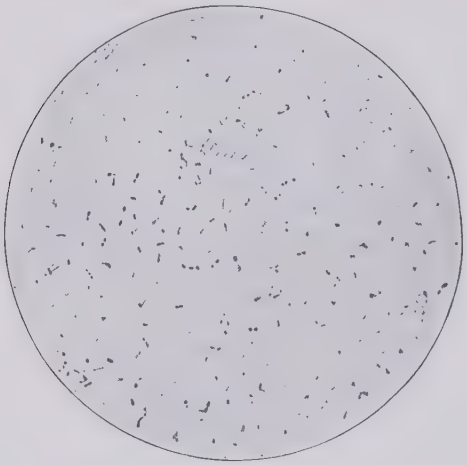


Fig. 169.—Bacillus of influenza (Muir and Ritchie).

Notwithstanding the number of examinations conducted to determine the cause of influenza, it was not until 1892, after the great epidemic of influenza, that Pfeiffer* found, in the blood and purulent bronchial discharges, a bacillus that conformed, in large part, to the requirements of specificity.

Morphology.—The bacilli (Fig. 169) are very small, having about the same diameter as the bacillus of mouse-

* "Deutsche med. Wochenschrift," 1892, 2; "Zeitschrift für Hygiene," 13.

septicemia, but only half its length (0.2 by 0.5 μ). They are usually solitary, but may be united in chains of three or four.

They are non-motile, and, so far as is known, do not form spores.

Staining.—They stain rather poorly, except with such concentrated and penetrating stains as carbol-fuchsin and Löffler's alkaline methylene-blue, and even with these more deeply at the ends than in the middle, so that they appear not a little like diplococci. They do not stain by Gram's method.

Canon * recommends a rather complicated method for the demonstration of the bacilli in the blood. The blood is spread upon clean cover-glasses in the usual way, thoroughly dried, and then fixed by immersion in absolute alcohol for five minutes. The best stain is Czenzynke's:

Concentrated aqueous solution of methylene-blue	40
0.5 per cent. solution of eosin in 70 per cent. alcohol	20
Distilled water	40

The cover-glasses are immersed in the solution, and kept in the incubator for three to six hours, after which they are washed in water, dried, and mounted in Canada balsam. By this method the erythrocytes are stained red, the leukocytes blue; and the bacilli, also blue, appear as short rods or as dumb-bells.

Large numbers of bacilli may be present, though sometimes only a few can be found after prolonged search. They are commonly inclosed within the leukocytes. It is scarcely necessary to pursue so tedious a staining method for demonstrating the bacilli, for they stain well enough for recognition by ordinary methods.

Isolation.—The influenza bacillus grows poorly upon artificial culture media, and is not easy to isolate, because the associated bacteria tend to outgrow it. When isolated it is difficult to keep, as it soon dies in unnatural environment.

Cultivation.—The bacillus does not grow in gelatin or upon ordinary agar-agar. Upon glycerin agar-agar, after twenty-four hours in the incubator, minute colorless, transparent, dewdrop-like colonies may be seen along the line of inoculation. They look like condensed moisture, and Kita-

* "Centralbl. f. Bakt.," etc., Bd. XIV, p. 860.

sato makes a special point of the fact that they never become confluent. The colonies may at times be so small as to require a lens for their detection.

In bouillon a scant development occurs, small whitish particles appearing upon the surface, subsequently sinking to the bottom and causing a "wooly" deposit there. The bacillus grows more luxuriantly upon culture media containing hemoglobin or blood, and can be transferred from culture to culture many times before losing vitality.

Vital Resistance.—Its resisting powers are very re-

Fig. 170.—Bacillus of influenza; colonies on blood agar-agar. Low magnifying power (Pfeiffer).

stricted, as it speedily succumbs to drying, and is certainly killed by an exposure to a temperature of 60° C. for five minutes. It will not grow at any temperature below 28° C.

Specificity.—From the fact that the bacillus is found chiefly in cases of influenza, that it is present as long as the purulent secretions of the disease last, and then disappears, and that Pfeiffer was able to demonstrate its presence in all cases of uncomplicated influenza, it seems that his conclusion that the bacillus is specific is justifiable. It is also found in the secondary morbid processes following influenza,

such as pneumonia, endocarditis, etc. Horder* has cultivated it from the valvular vegetation of two cases of endocarditis following influenza.

Pathogenesis.—The bacillus is pathogenic for very few of the laboratory animals, the guinea-pig being susceptible of fatal infection. The dose required to cause death of a guinea-pig varies considerably.

Immunity.—In the immunization experiments of Deline and Kole † one-twentieth of a twenty-four-hour-old culture was fatal in twenty-four hours. They found that the toxicity of the culture does not depend upon a soluble toxin, but upon an intracellular toxin. The outcome of the researches, which were made most painstakingly, was total failure to produce experimental immunity.

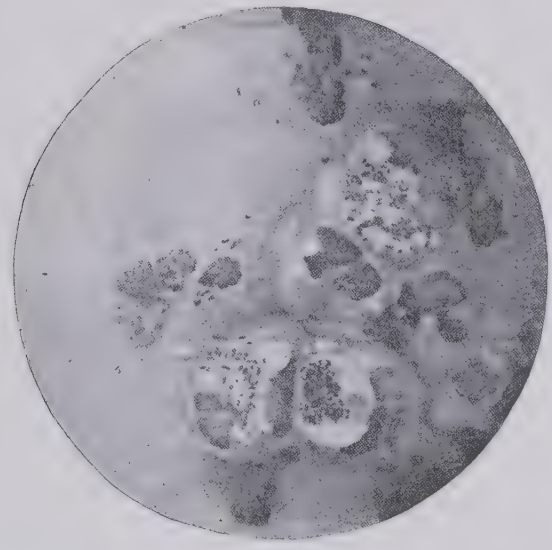


Fig. 171.—Bacillus of influenza; cover-glass preparation of sputum from a case of influenza, showing the bacilli in leukocytes. Highly magnified (Pfeiffer).

Increasing doses of the cultures, injected into the peritoneal cavity, enabled the animals to resist more than a fatal dose, but never enabled them to maintain vitality when large doses of living cultures were administered. This observation

* "Path. Soc. of Lond.," "Brit. Med. Jour.," April 22, 1905.

† "Zeitschrift für Hygiene," etc., Bd. XXIV, 1897, Heft 2.

is in exact harmony with the familiar clinical observation that, instead of an individual remaining immune after an attack of influenza, he is quite as susceptible as before.

A. Catanni, Jr.,* trephined rabbits and injected influenza toxin into their brains, at the same time trephining control animals, into some of whose brains he injected water. The animals receiving 0.5–1 mg. of the living culture died in twenty-four hours with all the nervous symptoms of the disease, dyspnea, paralysis beginning in the posterior extremities and extending over the whole body, clonic convulsions, stiffness of the neck, etc. Control animals injected in the same manner with water, and with a variety of other pathogenic bacteria never manifested similar symptoms. The virulence of the bacillus increased rapidly when transplanted from brain to brain.

Diagnosis of Influenza.—Wynekoop † employs for diagnosticating influenza and isolating the bacillus, a culture outfit similar to that used for diphtheria diagnosis, except that the serum contains more hemoglobin. The swab is used to secure secretions from the pharynx and tonsils, and from the bronchial secretions of patients with influenza, then rubbed over the blood-serum. In many such cultures minute colonies corresponding to those of the influenza bacillus were found. Those most isolated were picked up with a wire and transplanted to bouillon, from which fresh blood-serum was inoculated and pure cultures secured.

Carbol-fuchsin was found most useful for staining the bacilli. Wynekoop observed that influenza and diphtheria bacilli sometimes coexist in the throat, and that influenza bacilli are present in the sore eyes of those in the midst of household epidemics of influenza.

* "Zeitschrift für Hygiene," etc., 1896, Bd. XXIII.

† "Bureau and Division Reports," Department of Health, city of Chicago, Jan., 1899.

CHAPTER XIV.

ACUTE CONTAGIOUS CONJUNCTIVITIS.

The Koch-Weeks Bacillus.

General Characteristics.—A minute slender bacillus, non-motile, non-flagellated, non-sporogenous, non-liquefying, non-chromogenic, aerobic, and optionally anaerobic, staining by the ordinary methods but not Gram's method, susceptible of cultivation upon special media only and pathogenic for acute contagious conjunctivitis.

Acute contagious conjunctivitis is a common and world-wide affection, sometimes called "pink eye," and sometimes erroneously called catarrhal conjunctivitis. All its characteristics, and especially its contagiousness, point to its being a specific disease due to a specific cause, and thus entirely different from ordinary non-specific catarrh.

Specific Micro-organism.—The first bacteriologic investigation of acute contagious conjunctivitis was made by Robert Koch,* when in Egypt investigating a cholera epidemic. While in Alexandria he examined the secretions from fifty cases of conjunctivitis, finding the gonococcus, or an organism closely resembling it, in one form of the disease, and in a less severe form, a peculiar small bacillus. He seemed satisfied with this observation, or had no time to pursue the matter farther, for no cultivation or other experiments are mentioned.

The organism was observed from time to time, but no serious consideration seems to have been devoted to it until Weeks† published an account of what seemed to be the identical organism, which he not only observed, but also cultivated, and eventually successfully inoculated into the human conjunctival sac with the production of the disease. In the same year Kartulis‡ in Alexandria succeeded in cultivating the same organism. In 1894 Morax published a brochure in Paris in which he says that "the disease [which he describes under

* "Wiener klin. Wochenschr.," 1883, p. 1550.

† "N. Y. Med. Record," May 21, 1887.

‡ "Centralbl. f. Bakt. u. Parasitenk.," 1887, p. 289.

the name of acute conjunctivitis] is characterized by the constant presence in the conjunctival secretions of a small bacillus seen for the first time by Koch, but studied some years later by Weeks, and now known as the bacillus of Weeks."

Further descriptive and clinical information can be found in a paper by Weeks, "The Status of our Knowledge of the Ætiological Factor in Acute Contagious Conjunctivitis."*

Morphology.—The organism is said to bear some resemblance to the bacillus of mouse-septicemia. It measures 1 to 2 × 0.25 μ (Weeks). The length is more constant in

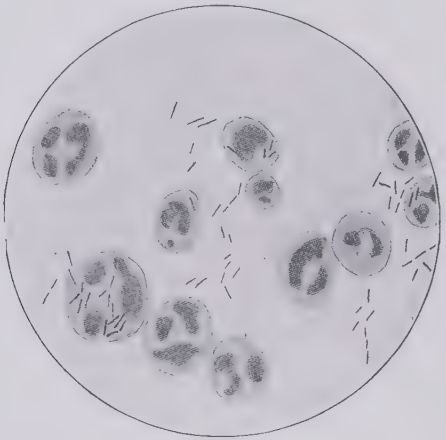


Fig. 172.—Koch-Weeks' bacillus from conjunctival exudate at third day of epidemic conjunctivitis (obj. Spencer one-twelfth oil-immersion) (Boston).

individuals found in the pus than those taken from cultures. In cultures the organisms sometimes form long chains, the division between the individuals being very indistinctly marked. No spores are observed. The organism has no flagella and is not motile.

Staining.—Weeks found that the organism stained well with watery solutions of methylene-blue, basic fuchsin or gentian violet. The color is fainter than that of the nuclei of the associated pus corpuscles, and is much less intense in old than in fresh cultures. It is readily given up when

* "New York Eye and Ear Infirmary Reports," vol. III, Part I, Jan., 1895, p. 24

treated with alcohol or acids. Morax found that the bacilli did not retain the color in Gram's method.

Cultivation.—The organism refuses to grow upon any of the ordinary culture media. Weeks found, however, that if the percentage of agar-agar used was reduced to 0.5 per cent., growths could be secured by incubation at 37° C., and successful transplantations carried on to the sixteenth generation. Abundant moisture was essential. The method of isolation adopted by Weeks was as follows:

"The conjunctival sacs were thoroughly washed with clean water, removing the secretion present by means of absorbent cotton. The patient was then directed to keep the eyes closed. After five or ten minutes had elapsed, the eyes were opened, and the secretion that had formed, lying at the bottom of the cul-de-sac, was removed by means of a sterilized platinum rod and transferred to the surface of the agar. The mass of tenacious secretion was drawn over the surface of the organ and left there, the platinum being thrust into the agar two or three times before removal."

At the end of forty-eight hours a slight haziness appears along the path of the wire, and on the surface of the agar a very small patch is noticeable; this is a pearly color and possesses a glistening surface. By the formation of small concentric colonies the growth extends for a short distance. At the end of the fourth or fifth day the growth ceases to advance; it is never abundant. The culture dies in from one to three weeks.

Pathogenesis.—Both Weeks and Morax have tested the organism for pathogenic activity, and in every case in which pure cultures of it were placed upon the human conjunctiva, typical attacks of the acute conjunctivitis resulted. The organism fails to infect any of the lower animals.

Association.—Both Weeks and Morax found the organism in intimate association with a larger club-shaped bacillus which was regarded as the pseudo-diphtheria bacillus. It seems to be of no pathogenic significance.

CHAPTER XV.

MALTA FEVER.

Micrococcus Melitensis (Bruce).

General Characteristics.—A non-motile, non-flagellate, non-sporogenous, non-chromogenic, non-liquefying, pathogenic coccus, staining by the ordinary methods, but not by Gram's method; characterized by remarkably slow growth and by pathogenic action upon monkeys.

In 1887, while working in Malta, Bruce * succeeded in finding in every fatal case of Malta fever a micrococcus which could be isolated in pure cultures from the spleen, liver, and kidney, which grew readily on artificial media, and which, when injected into monkeys, produced the disease. The serum from cases of Malta fever also caused agglutination of the cocci.

Morphology.—Micrococcus melitensis, as he called it, is a round or slightly oval organism measuring about 0.3 μ in diameter. It is usually single, sometimes in pairs, but never in chains. When viewed in the hanging drop, it is said to exhibit active "molecular" movements, but is not motile and has no flagella.

Staining.—It stains well with aqueous solutions of the anilin dyes, but not by Gram's method.

Cultivation.—The best medium for its cultivation is said to be ordinary agar-agar. After inoculating by a puncture, from an organ of a fatal case of Malta fever, the tubes should be kept at 37° C. No growth appears for several days. At length, however, minute pearly white spots appear scattered around the point of puncture and along the needle path. After some weeks the colonies grow larger and join to form a rosette-like aggregation, while the needle tract becomes a solid rod of yellow-brown color. After a lapse of months the growth still remains restricted to the same area and its color deepens to buff.

When the sloping surface of inoculated agar-agar is examined by transmitted light, the appearance of the colonies is somewhat different. At the end of nine or ten days, if

* "Practitioner," xxxiv, p. 161.

kept at 37° C., some of the colonies have a diameter of 2 to 3 mm. They are round in form, have an even contour, are slightly raised above the surface of the agar-agar, and are smooth and shining in appearance. On examining the colonies by transmitted light, the center of each is seen to be yellowish, while the periphery is bluish-white in color. The same colonies by reflected light appear milky white in color. Colonies on the surface of the agar-agar are found to be no larger than hemp-seed after a couple of months of cultivation.

When kept at 25° C., no colonies become visible to the

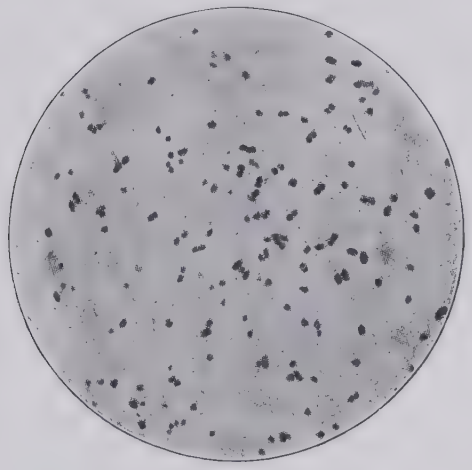


Fig. 173.—Micrococcus melitensis (Muir and Ritchie).

naked eye before the seventh day; at 37° C., before the third or fourth day.

Scarcely any growth takes place in gelatin, and no liquefaction of the medium occurs.

No growth takes place on boiled potato.

Plate cultures are not adapted to the study of the organism because of its extreme slowness of growth.

The thermal death-point given by Dalton and Eyre* is 57·5° C.

Pathogenesis.—The micro-organism is not pathogenic for mice, guinea-pigs, or rabbits, but is fatal to monkeys

* "Jour. of Hyg.," 1904, IV, p. 157.

when agar-agar cultures suspended in water are injected beneath the skin.

The micro-organism usually seems to be absent from the circulating blood, though Hughes has cultivated it from the heart's blood of a dead monkey.

Bruce not only succeeded in securing the micro-organism from the cadavers of Malta fever, but has also obtained it during life by splenic puncture.

Accidental inoculation with micrococcus melitensis, as by the prick of a hypodermic needle, is almost invariably followed by an attack of the disease. Six cases of this kind occurred in connection with bacteriologic work on Malta fever at Netley and two additional at the Royal Naval Hospital at Haslar and in the Philippines.*

The natural history of the micrococcus and the sources of contagion are unknown, and, as Bruce points out, would be very difficult to determine because of the high temperature at which its development takes place, the extreme slowness of its growth, and the absence of well-marked morphologic, cultural, or pathogenic characteristics by which it can be recognized.

* See Wright and Windsor, "Jour. of Hygiene," II, 1902, p. 413.

MISCELLANEOUS DISEASES.

SYMPTOMATIC ANTHRAX.

BACILLUS ANTHRACIS SYMPTOMATICI (BOLLINGER AND FESER).

General Characteristics.—A motile, flagellated, sporogenous, anaerobic, aerogenic, non-chromogenic, liquefying, pathogenic bacillus, that grows well in artificial culture media, infects the laboratory animals, and stains by the ordinary methods, though not by Gram's method.

"Symptomatic anthrax," *Charbon symptomatique du bœuf, Rauschbrand,* "quarter-evil," and "black-leg" are names applied to a peculiar disease of cattle common during the summer season in the Bavarian Alps, Baden, Schleswig-Holstein, and some parts of the United States, chiefly characterized by the occurrence of groups of irregular, emphysematous, crepitating, subcutaneous pustules, fever, wasting, and death. Diseased areas are also found in the muscles, and are most common over the quarters, hence the name "quarter-evil." When incised, the affected tissues have a dark color and contain a dark, bloody serum.

The micro-organismal nature of the disease had been suspected from an early date, but until the work of Bollinger and Feser * the disease was confounded with anthrax. Later, Arloing, Cornevin, and Thomas † studied the disease, and succeeded in demonstrating the specific micro-organism, which Kitasato ‡ first successfully cultivated.

Morphology.—The bacillus is large (3–5 μ in length, 0.5–0.6 μ in breadth), with rounded ends, is occasionally united in pairs, but never in chains (Fig. 174). It is motile when first examined in the hanging drop, but after a short time, perhaps because of the exposure to the oxygen in the hanging-drop preparations, the movement is lost and the bacilli die. When stained by Löffler's method, a considerable number of flagella can be demonstrated. Large oval spores are formed and by their presence distend the bacilli, causing them to assume a spindle or drumstick shape. Involution

* "Berliner thierärztliche Wochenschrift," 1878.

† "Compte-rendu de l'Acad. des Sciences de Paris," 1880–1883.

‡ "Zeitschrift für Hygiene und Infektionskrankheiten," Bd. VIII.

forms of the bacilli of enormous size and granular appearance, are common in old cultures.

Staining.—The bacillus can be stained with ordinary aqueous solutions of the anilin dyes, but not by Gram's method. It can be colored in sections of tissue with Löffler's solution, and can be observed in the blood without staining shortly after death.

The spores can be stained by ordinary methods. They are quite resistant to the action of heat and disinfectants, and withstand the effects of drying for a considerable length of time.

Cultivation.—The bacillus of symptomatic anthrax (Fig. 175) is strictly anaerobic. It grows at temperatures above

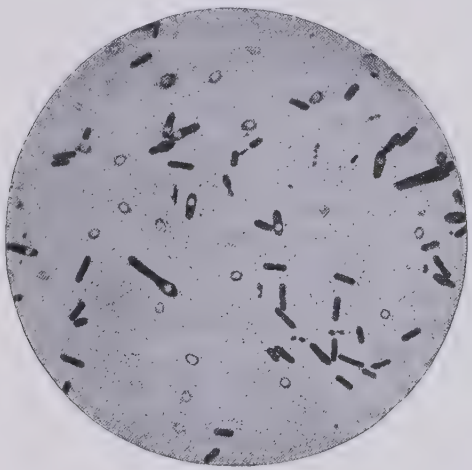


Fig 174.—Bacillus of symptomatic anthrax, containing spores, from an agar-agar culture. ×1000 (Fränkel and Pfeiffer).

18° C., but best at 37° C. The artificial cultivation, which was first achieved by Kitasato, is not more difficult than that of other anaerobic organisms.

Colonies.—When the bacteria are grown without oxygen in Esmarch tubes, the colonies are irregularly club-shaped or spheric, with a tangled mass of delicate projecting filaments visible upon microscopic examination.

Gelatin.—In gelatin containing 1 to 2 per cent. of glucose or 5 per cent. of glycerin the organism develops quite well, the exact appearance depending somewhat upon the method

of planting. If the bacteria be dispersed throughout the culture medium, the little colonies appear in the lower parts of the tube as nearly spheric or slightly irregular, clouded, liquefied areas containing bubbles of gas. If, on the other hand, the inoculation be made by a deep puncture, a stocking-shaped liquefaction forms along the whole lower part of the puncture, with considerable gas-production, and finally causes the liquefaction of all the gelatin except a thin superficial stratum. A peculiar acid odor is given off by the cultures.

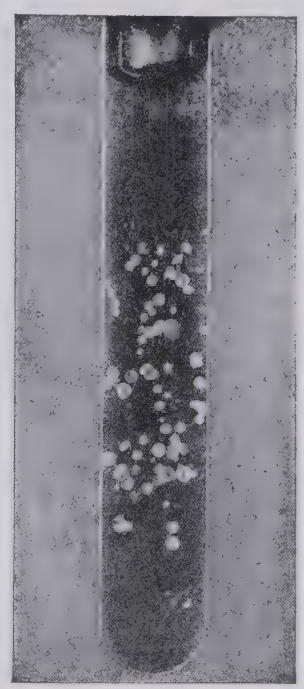


Fig. 175.—Bacillus of symptomatic anthrax; four-days-old culture in glucose gelatin (Fränkel and Pfeiffer).

Agar-agar.—In agar-agar the development is similar to that in gelatin. The gas-production is marked, the liquefaction of course absent, and the same acid odor pronounced.

Bouillon.—The bacillus also develops quite well in bouillon, the bacillary masses sinking to the bottom in the form of whitish flakes, while the gas bubbles collect at the top. In this medium the virulence is unfortunately soon lost.

Milk.—The development of the bacilli in milk is unaccompanied by coagulation.

Pathogenesis.—When susceptible animals are inoculated with a minute portion of a pure culture, the bacilli grow and produce the well-known affection, with its fatal outcome. Cattle seem to be the most susceptible animals, especially when between six months and four years old; sheep and goats are also sometimes affected. Curiously enough, animals that are immune against malignant edema seem to be more susceptible than others to "Rauschbrand." Of the laboratory animals, the guinea-pig is most susceptible; swine, dogs, and rabbits are slightly susceptible; horses, goats, and birds are immune.

When the guinea-pig is inoculated with the bacillus of symptomatic anthrax, it dies in from twenty-four to thirty-six hours. The post-mortem examination shows bloody serum at the point of inoculation, and dark red or black muscular tissue like that of the "black-leg" of cattle. No changes are apparent in the internal organs. The bacilli are at first found near the point of inoculation in the inflammatory exudations only, but soon after death, being motile, they spread to all parts of the body.

Virulence.—The virulence of the organism is soon lost in all culture media, but it is said that lost virulence can be brought back and existing virulence much increased by the addition of 20 per cent. of lactic acid to the culture.

Vaccination.—The virulence of the bacillus can easily be attenuated by exposure to heat, by exposure of its spores to heat, or by drying combined with exposure to heat. Inoculation of animals with attenuated bacilli causes a mild local affection, followed by complete immunity against the virulent organisms. Upon this principle the prophylactic vaccination is based. Kitt* has shown that dried muscle from an infected animal is more efficacious for the purpose of immunizing than cultures of the bacilli.

The method of preparing the so-called "vaccines" is very simple. A calf is inoculated in the muscular tissue with a virulent culture. As soon as it dies, the black, spongy muscular tissue is dissected out, cut into small pieces, and dried in an oven at about the body temperature. When dry, the muscle is ground to a coarse powder, then heated for the purpose of attenuating the virulence of the contained bacilli. Two inoculations, one of pulverized muscle heated for six or seven hours to 100°–104° C., and a week later one heated to 85°–90° C., were originally used, but most observers are now agreed that a single injection of muscle attenuated by six or seven hours' exposure to 85° C. will suffice for immunization. The muscle-powder is simply crushed in a mortar, in a convenient quantity of sterile water, and injected hypodermically.

The statistics of Guillod and Simon, based upon 3500 protective inoculations, show a death-rate from 5–20 per cent. in unprotected animals and 0.5–2 per cent. in protected animals.

Immunity against symptomatic anthrax seems, however, to be one of degree, for Arloing, Cornevin, and Thomas found that when the bacillus is introduced simultaneously with a 20 per cent. solution of lactic acid, either the virulence of

* "Centralbl. f. Bakt., Parasitenk., u. d. Infektionskrankh.," Bd. III, Nos. 18 and 19.

the bacillus or the resistance of the tissues was so changed that natural immunity was destroyed and the bacteria allowed to develop and produce the disease. Roger found also that refractory animals, like the rabbit, mouse, pigeon, and chicken, could be made susceptible by combined injection of "Rauschbrand" bouillon and Bacillus prodigiosus, Proteus vulgaris, or other harmless organisms.

It may be that some of the accidents attending the use of the prophylactic depend upon a mixed infection by which the virulence of the bacillus of symptomatic anthrax is increased.

The bacteria probably enter the animal in spontaneous infection through punctures or fly-bites. Epidemics commonly arise at certain geographic points, known technically as "Rauschbrand stations."

Sanitation.—At first thought, as Fränkel points out, one might imagine that an animal dead of quarter-evil and the discharges from its body might be harmless, as compared, for example, with the cadavers and discharges of anthrax, because of the purely anaerobic growth of the bacillus and the rapidity of its death in the presence of oxygen. This is, however, untrue, for the highly resisting spores make the pollution of the soil exceedingly dangerous for cows subsequently browsing upon it. That the spores are of great vitality is shown by the well-known laboratory method of keeping them on hand for experimental purposes, dried in the muscular tissue of a diseased animal.

Every precaution should be exerted to have affected animals isolated, and their cadavers disinfected and destroyed, or buried in such a manner that subsequent infection shall be impossible.

MALIGNANT EDEMA.

Bacillus Œdematis Maligni (Koch).

General Characteristics.—A motile, flagellated, sporogenous, anaerobic, liquefying, aerogenic, non-chromogenic, pathogenic bacillus of the soil, readily stained by the ordinary methods, but not by Gram's method.

This organism was originally found by Pasteur* in putrescent animal infusions and called by him (1875) Vibrion septique. It was later more carefully studied and described by Koch.†

Distribution.—The organism is widely distributed in nature, being commonly present in garden earth. It is also found in dust, in waste water from houses, and sometimes in the intestinal canals of animals.

Morphology.—The bacillus of malignant edema is a large rod-shaped organism with rounded ends, measuring 2–10 μ by 0.8–1.0 μ. It is actively motile, and possesses many

Fig. 176.—Bacillus of malignant edema, from the body-juice of a guinea-pig inoculated with garden earth. × 1000 (Fränkel and Pfeiffer).

flagella. It produces oval endospores centrally situated without alteration in the shape of the parent bacillus.

Staining.—The bacillus stains well with ordinary cold aqueous solutions of the anilin dyes, but not by Gram's method.

Cultivation.—The organism grows well both at the room temperature and at that of the incubator. It is not difficult to secure in pure culture, being most easily obtained from the edematous tissues of guinea-pigs and rabbits inoculated with garden earth.

* "Bull. Acad. Méd.," 1877 and 1881.

† "Mittheilungen aus dem kaiserl. Gesundheitsamte," I, 53.

Colonies.—The colonies which develop upon the surface of gelatin kept under anaerobic conditions appear to the naked eye as small shining bodies with liquid, grayish-white contents. Under the microscope they appear filled with a tangled mass of long filaments which under a high power exhibit active movement. The edges of the colony have a fringed appearance, much like the colonies of the hay or potato bacillus.

In gelatin tube cultures the characteristic growth cannot be observed in a puncture, because of the air which remains in the path of the wire, unless the tube be placed under anaerobic conditions. The best preparation, therefore, is made by heating the gelatin to expel any air it may contain, inoculating it while still liquid, and solidifying it in cold (iced) water. In such a tube the bacilli develop in globular circumscribed areas of cloudy liquefaction (Fig. 177), which contain a small amount of gas. In gelatin to which a little grape-sugar has been added the gas-production is marked. The gas is partly inflammable, partly not. A distinct odor accompanies the gas-production, and is especially noticeable in agar-agar cultures.

Metabolic Products.—Of the toxic products of the organism nothing definite is known. It decomposes albumin, forming fatty acids, leucin, hydroparacumaric acid, and an oil with an offensive odor. Among the gases formed carbonic acid, hydrogen, and marsh gas have been detected.

Pathogenesis.—When introduced beneath the skin, the bacillus is pathogenic for a large number of animals—mice, guinea-pigs, rabbits, horses, dogs, sheep, goats, pigs, calves, chickens, and pigeons. Cattle seem to be immune.

Günther points out that the simple inoculation of the bacillus upon an abraded surface is insufficient to produce infection, because the presence of oxygen is detrimental to its growth. When the bacilli are deeply introduced beneath the skin, infection occurs.

Mice, guinea-pigs, and rabbits sicken and die in about forty-eight hours.

Lesions.—In the blood the bacilli are few because of the loosely combined oxygen it contains. The great majority of the bacilli occupy the subcutaneous tissue, where very little oxygen is present and the conditions of growth are good. The autopsy shows a marked subcutaneous edema containing immense numbers of the bacilli. If the animal

be permitted to remain undisturbed for some time after death, the bacilli spread to the circulatory system and reach all the organs.

Brieger and Ehrlich* have reported two cases of malignant edema in man. Both occurred in typhoid fever patients subcutaneously injected with musk, the infection no doubt resulting from impurities in the therapeutic agent.

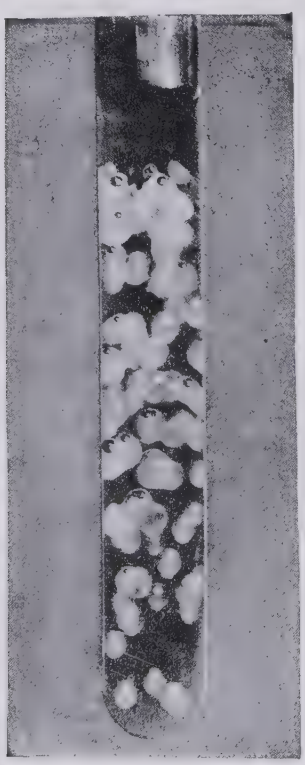


Fig. 177.—Bacillus of malignant edema growing in glucose gelatin (Fränkel and Pfeiffer).

Grigorjeff and Ukke† have observed another interesting case of typhoid fever with intestinal ulcerations, through which infection by the bacillus of malignant edema took place. The case was characterized by interstitial emphysema of the subcutaneous tissue of the neck and breast, gas bubbles in the muscles, and a transformation of the entire liver into a spongy, porous mass of a gray-brown color. The spleen was enlarged and soft, and contained a few gas bubbles. Though the writers consider this organism to be the bacillus of malignant edema, the general impression one receives from the description of the lesions suggests that it was Welch's Bacillus aerogenes capsulatus.

No case is reported in which healthy men have been infected with malignant edema.

Immunity.—Cornevin found that the passage of the bacillus through white rats diminished its virulence, and that the animals of various species that recovered were immune against the virulent organisms.

* "Berliner klin. Wochenschrift," 1882, No. 44.

† "Militär-medizin. Jour.," 1898, p. 323.

GASEOUS EDEMA.

Bacillus Aerogenes Capsulatus (Welch).

General Characteristics.—A large, stout, non-motile, non-flagellated, sporogenous, non-chromogenic, purely anaerobic, markedly aerogenic, doubtfully pathogenic bacillus, easily cultivated in artificial media, readily stained by the ordinary methods and by Gram's method.

This disease is caused by an interesting micro-organism described by Welch, and subsequently studied by Welch and Nuttall,* Welch and Flexner,† and others. Welch said at the meeting of the Society of American Bacteriologists held at Philadelphia, December 30, 1904, that he believed this organism to be identical with Kline's Bacillus enteritidis sporogenes, and that it belongs to the butyric acid group. It is probably also identical with Bacillus phlegmone emphysematose of Fränkel.‡ It was first secured by Welch from the body of a man dying suddenly of aortic aneurysm with a peculiar gaseous emphysema of the subcutaneous tissues and internal organs, and a copious formation of gas in the blood-vessels. The blood was thin and watery, of a lac color, and contained many large and small gas bubbles, and many bacilli, which were also obtained from it and the various organs, especially in the neighborhood of the gas bubbles, in nearly pure culture. The coloring-matter of the blood was dissolved out of the corpuscles and stained the tissues a deep red.

Distribution.—The organism is apparently of wide distribution. It is believed that the natural habitat of the bacillus is the soil, but there is reason to think that it commonly occurs in the intestine, and may occasionally be found upon the skin.

Morphology.—The bacillus is a large organism, measuring 3–5 μ in length, about the thickness of the anthrax bacillus, with ends slightly rounded, or, when joined, square (Fig. 178). It occurs chiefly in pairs and in irregular groups, but not in chains, in this particular differing from the anthrax bacillus. In culture media it is usually straight, with slightly rounded ends. In old cultures the rods may be slightly bent, and involution forms occur. The bacillus varies some-

* "Bull. of the Johns Hopkins Hospital," July and Aug., 1892, vol. VIII, No. 24.

† "Jour. of Experimental Medicine," Jan., 1896, vol. I, No. 1, p. 6.

‡ "Centralbl. f. Bakt.," etc., 1893, Bd. XIII, p. 13.

what in size, especially in length, in different culture media. It usually appears thicker and more variable in length in artificial cultures than in the blood of animals.

The bacillus is not motile and has no flagella. Endospores are formed upon Löffler's blood-serum.

It was at first thought that the bacillus produced no spores, but Dunham* found that spores were produced upon blood-serum, and especially upon Löffler's blood-serum bouillon mixture. The spores resist desiccation and exposure to the air for ten months. They stain readily in hot solutions of fuchsin in anilin water, and are not decolorized by a moderate exposure to the action of 3 per cent. solution of hydrochloric acid in absolute alcohol. They are oval, and are usually situated near the middle of the bacillus, which is distended because of the large size of the spore and bulges at the sides.

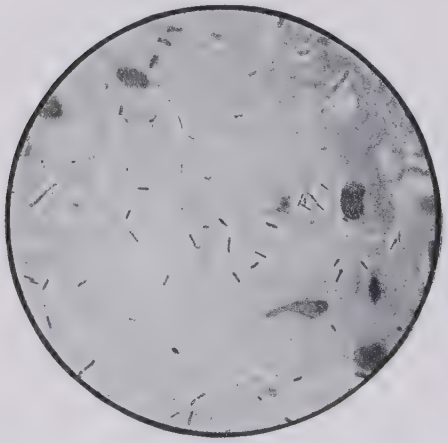


Fig. 178.—Bacillus aerogenes capsulatus (from photograph by Prof. Simon Flexner).

Staining.—The organism stains well with the ordinary stains, and retains the color well in Gram's method. When stained with methylene-blue a granular or vacuolated appearance is sometimes observed, due to the presence of unstained dots in the cytoplasm.

Usually in the body-fluids and often in cultures the bacilli are surrounded by distinct capsules—clear, unstained zones. To demonstrate this capsule to the best advantage, Welch and Nuttall devised the following special stain:

A cover is thinly spread with the bacilli, dried, and fixed without overheating. Upon the surface prepared, glacial acetic acid is dropped for a few moments, then allowed to drain off, and at once replaced by a strong aqueous solution

* "Bull. of the Johns Hopkins Hospital," April, 1897, p. 68.

of gentian violet, which is poured off and renewed several times until the acid has been replaced by the stain. The specimen is then examined in the coloring solution, after soaking up the excess with filter paper, the thin layer of coloring fluid not interfering with a clear view of the bacteria and their capsules. After mounting in Canada balsam the capsules are not nearly so distinct. The width of the capsule varies from one-half to twice the thickness of the bacillus. Its outer margin is stained, leaving a clear zone immediately about the bacillus.

The bacillus is *anaerobic* and *aerogenic*. It grows upon all culture media at the room temperature, though better at the temperature of incubation.

Cultivation.—Gelatin.—It grows in ordinary neutral or alkaline gelatin, but better in gelatin containing glucose, in which the characteristic gas-production is marked. Soft media, made with 5 instead of 10 per cent. of the crude gelatin, is said to be better than the standard preparation.

There is no distinct liquefaction of the medium, but in 5 per cent. gelatin softening can sometimes be demonstrated by tilting the tube and observing that the gas bubbles change their position, as well as by noticing that the growth tends to sediment.

Fig. 179.—Bacillus aerogenes capsulatus, with gas-production (from photograph by Prof. Simon Flexner).

Agar-agar.—In making agar-agar cultures careful anaerobic precautions must be observed. The tubes should contain considerably more than the usual quantity of the medium, which should be boiled and freshly solidified before using. The implantation should be deeply made with a long wire. The growth takes place slowly unless such tubes are placed in a Buchner's jar or

other anaerobic device. The deeper colonies are the largest. Sometimes the growth only takes place within 10–12 mm. of the surface; at others, within 3–4 cm. of it. After repeated cultivation the organisms seem to become accustomed to the presence of oxygen, and will grow higher up in the tube than when freshly isolated.

Colonies.—The colonies seen in the culture medai are grayish-white or brownish-white by transmitted light, and sometimes exhibit a central dark dot. At the end of twenty-four hours the larger colonies do not exceed 0.5–1.0 mm. in diameter, though they may subsequently attain a diameter of 2–3 mm. or more. Their first appearance is as little spheres or ovals, more or less flattened, with irregular contours, due to the presence of small projecting prongs, which are quite distinct under a lens. The colonies may appear as little irregular masses with projections.

After several days or weeks, single, well-shaped colonies may attain a large size and be surrounded by projections, either in the form of little knobs or spikes or of fine branchings—hair-like or feathery. Their appearance has been compared to thistle-balls or powder-puffs and to thorn-apples. When the growth takes place in the puncture, the feathery projections are continuous. Bubbles of gas make their appearance in plain agar as well as in sugar-agar, though, of course, less plentifully. They first appear in the line of growth; afterward throughout the agar, often at a distance from the actual growth. Any fluid collecting about the bubbles or at the surface of the agar-agar may be turbid from the presence of bacilli. The gas-production is more abundant at 37° C. than at the room temperature.

The agar-agar is not liquefied by the growth of the bacillus, but is often broken up into fragments and forced into the upper part of the tube by the excessive gas-production.

In its growth the bacillus produces considerable acid.

Bouillon.—In bouillon growth does not occur in tubes exposed to the air, but when the tubes are placed in Buchner's jars, or kept under anaerobic conditions, it occurs with abundant gas-formation, especially in glucose-bouillon, with the formation of a frothy layer on the surface. The growth is rapid in development, the bouillon becoming clouded in two to three hours. After a few days the bacilli sediment and the bouillon again becomes clear. The reaction of the bouillon becomes strongly acid.

Milk.—In milk the growth is rapid and luxuriant under anaerobic conditions, but does not take place in cultures exposed to the air. The milk is coagulated in from twenty-four to forty-eight hours, the coagulum being either uniform or firm, retracted, and furrowed by gas bubbles. When litmus has been added to the milk, it becomes decolorized when the culture is kept without oxygen, but turns pink when it is exposed to the air.

Potato.—The bacillus will also grow upon potato when the tubes are inclosed in an anaerobic apparatus. There is a copious gas-development in the fluid at the bottom and sides of the tube, so that the potato becomes surrounded by a froth. After complete absorption of the oxygen a thin, moist, grayish-white growth takes place upon the surface of the medium.

Vital Resistance.—The vital resistance of the organism is not great. Its thermal death-point was found to be 58° C. after ten minutes' exposure. Cultures made by displacing the air with hydrogen are less vigorous than those in which the oxygen is absorbed from the air by pyrogallic acid. It was found that in the former class of cultures the bacillus died in three days, while in the absorption experiments it was kept alive at the body temperature for one hundred and twenty-three days. It is said to live longer in plain agar than in sugar-agar. To keep the cultures alive it has been recommended to seal the agar-agar tube after two or three days' growth.

Pathogenesis.—The pathogenic powers of the bacillus are limited, and while in some infected cases it seems to be the cause of death, its power to do mischief in the body seems to depend entirely upon the pre-existence of depressing and devitalizing conditions predisposing to its growth.

Being anaerobic, the bacilli are unable to live in the circulating blood, though they grow in old clots and in cavities, such as the uterus, etc., where little oxygen enters, and from which they enter the blood and are distributed.

In support of these views Welch and Nuttall show that when a healthy rabbit is injected with 2.5 c.c. of a fresh sugar-bouillon into the ear-vein, it usually recovers without any evident symptoms. After similar injection with but 1 c.c. of the culture, a pregnant rabbit carrying two dead embryos, died in twenty-one hours. It seems that the bacilli were first able to secure a foothold in the dead em-

bryos, and there multiplied sufficiently to bring about the subsequent death of the mother.

After death, when the blood is no longer oxygenated, the bacilli grow rapidly, with marked gas-production, which in some cases is said to cause the body to swell to twice its natural size. The effect upon guinea-pigs does not differ from that upon rabbits, though gaseous phlegmons are sometimes produced.

Pigeons, when subcutaneously inoculated in the pectoral region, frequently succumb. Following the injection gas-production causes the tissues of the chest to become emphysematous. The birds usually die in from seven to twenty-four hours, but may recover.

Intraperitoneal inoculation of animals sometimes causes fatal purulent peritonitis.

Sources of Infection.—The infection seen in man usually occurs from wounds into which earth has been ground, as in the case of a compound, comminuted fracture of the humerus, with fatal infection, reported by Dunham, or in wounds and injuries in the neighborhood of the perineum.

Among the twenty-three cases reported by Welch and Flexner * we find wounds of the knee, leg, hip, and forearm, ulcer of the stomach, typhoid ulcerations of the intestine, strangulated hernia with operation, gastric and duodenal ulcer, perineal section, and aneurysm, as conditions in which external or gastro-intestinal infection occurred.

Dobbin,† P. Ernst,‡ Graham, Stewart and Baldwin,§ and Krönig and Menge‖ have studied cases of puerperal sepsis and sepsis following abortion either caused by the bacillus, or in which it played an important rôle.

Williams ** has found the bacillus in a case of suppurative pyelitis.

The symptoms following infection are quite uniform, consisting of redness and swelling of the wound, with rapid elevation of temperature and rapid pulse. The wound usually becomes more or less emphysematous, and discharges a thin, dirty, brownish, offensive fluid that contains gas bubbles

* "Journal of Experimental Medicine," vol. I, No. 1, Jan., 1896.
† "Bull. Johns Hopkins Hospital," Feb., 1897, No. 71, p. 24.
‡ "Virchow's Archiv," Bd. CXXXIII, Heft 2.
§ "Columbus Med. Jour.," Aug., 1893.
‖ "Bakteriologie des weiblichen Genitalkanals," Leipzig, 1897.
** "Bull. Johns Hopkins Hospital," April, 1896, p. 66.

and is sometimes frothy. The patients occasionally recover, especially when the infected part can be amputated, but death is the common outcome. After death the body begins to swell almost immediately and may attain twice its normal size and be unrecognizable. Upon palpation a peculiar crepitation can be felt in the subcutaneous tissue nearly everywhere, and the presence of gas in the blood-vessels is easy of demonstration. The gas is inflammable, and as the bubbles ignite explosive sounds are heard.

At the autopsy the gas bubbles are found in most of the internal organs, sometimes so numerously as to justify the German term "Schaumorgane" (frothy-organs). The liver is especially apt to show this condition. When such tissues are hardened and examined microscopically, the bubbles appear as spaces in the tissue, their borders lined with large numbers of the bacillus. There are also clumps of bacilli without gas bubbles, but surrounded by tissue, whose nuclei show a disposition to fragment or disappear, and whose cells and fibers show signs of disintegration and fatty change. In discussing these changes Ernst concluded that they were ante-mortem and due to the irritation caused by the bacillus. The gas-production he regards as post-mortem.

In the internal organs the bacillus is usually found in pure culture, but in the wound it is usually mixed with other bacteria. On this account it is difficult to estimate just how much of the damage before death depends upon the activity of the gas bacillus. That gas-production after death has nothing to do with pathogenesis during life is shown by injecting into the ear-vein of a rabbit a liquid culture of the gas bacillus, permitting about five minutes' time for the distribution of the bacilli throughout the circulation, and then killing the rabbit. In a few hours the rabbit will swell and its organs and tissues be riddled with the gas bubbles.

At times, however, as in a case of Graham, Stewart and Baldwin, there is no doubt but that the bacillus produces gas in the tissues of the body during life. These observers, in a case of abortion with subsequent infection, found the patient "emphysematous from the top of her head to the soles of her feet" several hours before death.

In this case, in which the bacillus was found in pure culture, it would indeed be difficult to doubt that the fatal issue was due to Bacillus aerogenes capsulatus.

Probably the best review of the subject is to be found in "A Contribution to the Knowledge of the Bacillus Aerogenes Capsulatus," by W. T. Howard, Jr.*

PROTEUS INFECTION.

Bacillus Proteus Vulgaris (Hauser).

General Characteristics.—An actively motile, flagellated, non-sporogenous, non-chromogenic, liquefying, aerobic and optionally anaerobic, doubtfully pathogenic, aerogenic bacillus, easily cultivated on artificial media and readily stained by the ordinary methods, though not by Gram's method.

This bacillus was first found by Hauser † in decomposing animal infusions, usually in company with two closely allied forms, Proteus mirabilis and Proteus zenkeri, which, as the experiments and observations of Sanfelice and others show, may be identical with it. According to Kruse, it is quite probable that the mixed species formerly called *Bacterium termo* was largely made up of the proteus.

Distribution.—The *proteus* has been secured in cultures from wound and puerperal infections, purulent peritonitis, endometritis, and pleurisy. When the local lesion is limited, as in endometritis, the danger of toxemia is slight; but when widespread, as the peritoneum, it may prove serious. Bacillus proteus has also been found in acute infectious jaundice and in acute febrile icterus, or Weil's disease.

Morphology.—The bacilli are variable in size and shape—pleomorphic—and are named *proteus* from this peculiarity. Some differ very little from cocci, some are more like the colon bacillus in shape, others form long filaments, and occasional spirulina forms are met with. True spirals are never found. All of the forms mentioned may be found in pure cultures of the same organism. The diameter of the bacillus is usually about 0.6 μ, but the length varies from 1.2 μ or less to 4 μ or more. No spores are formed. The organisms are actively motile. The long filaments frequently form loops and tangles. Flagella are present in large numbers. Upon one of the long bacilli as many as one hundred have been counted. Involution forms are frequent in old cultures.

* "Contributions to the Science of Medicine by the Pupils of W. H. Welch," 1900, p. 461.

† "Ueber Faulnissbakterien," Leipzig, 1885.

Staining.—The bacilli stain well by the ordinary methods. Gram's method usually fails.

Cultivation.—The proteus is easily cultivated and grows well in all the artificial media.

Colonies.—Upon gelatin plates a typical phenomenon is observed in connection with the development of the colonies, for the most advantageous observation of which the medium used for making the cultures should contain 5 instead of 10 per cent. of gelatin. Kruse * describes the phenomenon as follows: "At the temperature of the room, rounded, saucer-shaped depressions, with a whitish central mass surrounded by a lighter zone, are quickly formed. Under low magnification the center of each is seen to be surrounded by radiations extending in all directions into the solid gelatin, and made up of chains of bacilli. Between the radiations and the granular center bacteria are seen in active motion. Upon the surface the colony extends as a thin patch, consisting of a layer of bacilli arranged in threads, sending numerous projections from the periphery. Under certain conditions the wandering of the processes can be directly observed under the microscope. It depends not only upon the culture medium, but, in part, upon the culture itself. Entire groups of bacilli or single threads, by gradual extension and circular movement, detach themselves from the colony and wander about upon the plate. From the radiated central part of the colony peculiar zooglea are formed, having a sausage or screw shape, or wound in spirals like a corkscrew. The

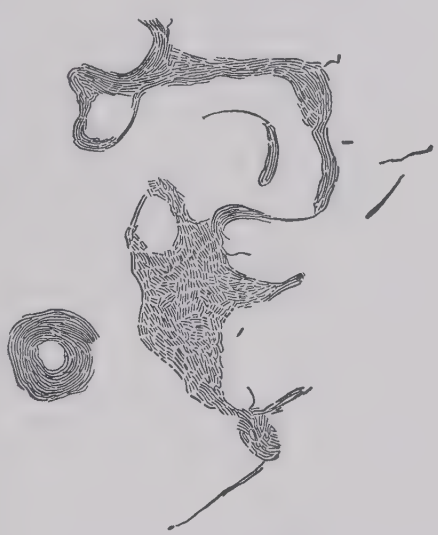


Fig. 180.—Swarming islands of proteus bacilli on the surface of gelatin; × 650 (Hauser).

* Flügge's "Die Mikroorganismen."

younger colonies, which have not yet reached the surface of the gelatin, are more compact, rounded or nodular, later covered with hair-like projections, and becoming radiated like the superficial colonies."

If the culture medium be concentrated, or the culture has been frequently transplanted, the phenomenon is less marked and may not occur.

Gelatin Punctures.—Puncture cultures in gelatin are not characteristic. A stocking-like liquefaction of the gelatin extends so rapidly that the entire gelatin is liquefied in a few days. Anaerobic cultures do not liquefy.

Agar-agar.—Upon agar-agar the bacillus forms a moist, thin, transparent, rapidly extending layer which rarely reaches the sides of the tube. Upon agar-agar plates ameboid movement of the colonies may also occur.

Potato.—Upon potato the growth occurs in the form of a smeary patch of soiled appearance.

Milk.—Milk is coagulated.

Metabolic Products.—The bacillus usually produces alkalies. Indol and phenol are formed from the peptone of the culture media. Nitrates are reduced to nitrites, and then partly reduced to ammonia. In most culture media not containing sugar the bacillus produces a very disagreeable odor.

In culture media containing either grape- or cane-sugar fermentation occurs both in the presence and in the absence of oxygen. Milk-sugar is not decomposed.

Pathogenesis.—It is a question whether or not Bacillus proteus is to be ranked among the pathogenic bacteria. Small doses are harmless for the laboratory animals; large doses produce abscesses. A toxic substance resulting from the metabolism of the organism seems to be the cause of death when considerable quantities of a culture are injected into the peritoneal cavity or blood-vessels. The bacilli do not seem able to multiply in the healthy animal body, but can do so when previous disease or injury of its tissues has taken place.

Bordoni-Uffreduzzi has shown that the proteus quite regularly invades the tissues after death though it appears unable to maintain an independent existence in the tissues during life, and is probably of importance only when present in association with other bacteria. It at times grows abundantly in the urine and may produce primary

inflammation of the bladder when introduced spontaneously or experimentally into that viscus. The inflammatory process may also extend from the bladder to the kidney, and so prove quite serious.

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